Chapter 1:

Introduction

1- Introduction

1.1- General introduction

Sequential extraction of solid samples such as soils and sediments has been applied for several years in order to increase knowledge about the bonding of metals in such samples. As interest in the environment impact of metals became more important during the 1970s, the need to study the distributions of heavy metals in soils has increased. Heavy metal pollution of water can have a critical impact on plant and animal life. The soil may act as the reservoir for the release of the heavy metals into waterways; depending on changes in the environment, heavy metals can leach out of soil and into waterways spreading the contamination for several years. As well, vegetation grown in metals polluted soils can take up some of the metals and pass the contamination through the food chain. Finally, it is necessary to know how heavy metals are retained by soils in order to remediate sites. Sequential extraction scheme is a primary tool for this objective.

1.1.1- Soil Pollution

Soil is made up several different components including minerals, organic matter, gases, and soil pore-water. The solid fraction of the soil can make up anywhere from 50 % to 75 % of the soil mass. The organic matter can change from 1 % to 30 % depending on the environmental conditions. For the adsorption of heavy metals, the solid soil components are very important, although the soil pore-water allows for the movement of the metals through soil. The mineral fraction of the soil is mainly weathered material from the parent rock and therefore can be different from one region to another according to the mineral circulation of a geographic region. There are various types of soil minerals which have been further sub-classified as primary silicates, secondary silicates, clay minerals, oxides, hydroxides,

carbonates and sulfates. Depending on the physico-chemical properties of a metal, it can absorb onto any one of the several soil components.

1.1.2- Mobility potential

Sequential extraction is the chosen extraction method for the removal of various components of the soil and the trace elements which are bound to these components. Since most analytical instruments can only analyze samples in liquid form, the extraction of the components into solution is essential.

On the other hand, the complex and changeable organization of soils may pose problems in the extraction of metals from soil such as redistribution of the metal variety onto their soil components. Using standard reference materials can shed light on the redistribution of metals during extractions and on the totality of the extraction procedure. In this way, a sequential extraction procedure with minimal redistribution property can be developed.

One such sequential extraction procedure is the extensively used approach first developed by (Tessier *et al.* 1979). The BCR (now the Standards, Measurements and Testing programme of the European Commission) sequential extraction scheme (Ure *et al.* 1993) is the latest development towards an internationally standardized method for geochemical analysis of solid samples. An improved version of the latter scheme was applied to sludge samples obtained from shrimp aquaculture sludge and sediment samples from Sungai Buloh Selangor Malaysia (Nemati *et al*., 2009). In this work, the methods of instrumental analysis were Flame Atomic Absorption spectrometry (FAAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), both of which require the sample to be in liquid form. For solid materials, sample digestion was required and alternative pseudo-total digestion methods were tested for this purpose. For analytical quality guarantee and

procedural confirmation, standard reference materials (CRM) of known composition were processed in the same manner as the soils, permitting a rigorous estimation of the reliability of the extraction results.

1.1.3- Problem statement and limitations

As seafood is one of the major foods in eastern south of Asia, there are a lot of shrimp aquaculture farms in this region. At the end of each period of shrimp aquaculture, the resulted sludge is exposed to the air to get dried. The dried sludge is transferred to other places to be used as a fertilizer. It not only can contaminate these regions, but also can in different condition release various trace elements in environment. In this research project, we selected a shrimp aquaculture in Selangor, Malaysia and took four different samples in that site to investigate the mobility of heavy metals in shrimp aquaculture sludge. Also this work was done in Sungai Buloh to compare the results obtained by using the modified BCR sequential extraction methods in different sediment depths for discussion of mobility potential of heavy metals in depth.

1.2- Goals and Objectives

The first target of this work was to characterize trace metal contamination in selected sludge, soils and sediments collected from Selangor Malaysia. For this study, we selected a sample from shrimp aquaculture sludge as a first sample selected for sequential extraction scheme. As seafood is one of the major foods in Southeast Asian region, therefore, there are many shrimp aquaculture farms to be found in this region. After each harvesting period, the sludge is removed in order to maintain water quality of the pond. This sludge may be used as a fertilizer if the mobility of metals in the sludge is well-understood. The second sample was sediments from Sungai Buloh and the straits of Melaka, collected at several stations

and different levels. Specific objectives were the identification and quantification of selected heavy metals, and the assessment of metal partitioning and retention within the sludge and sediment.

The Second goal of this project was to modify the conventional sequential extraction procedures (Tessier and BCR) as to overcome of the main limitations of sequential extraction procedures that they are extremely time-consuming, and are less used for routine analysis. This problem has also been noted and is replacing the conventional procedure by other alternatives, such as microwave (MW) heating and ultrasonic (US) shaking.

This work will also compare the results obtained by using the conventional BCR sequential extraction methods with those from single extractions, employing similar operating conditions and using time-saving extraction devices (US bath and MW oven) on sediment samples. Sediment certified reference material BCR 701 was also employed to test the extraction efficiency of the accelerated procedures. The contents of Cd, Cu, Cr, Ni, Pb and Zn in the extracts were measured by flame atomic absorption spectrometry (FAAS). The advantages and disadvantages of the time-saving procedures were considered and evaluated from statistical and environmental points of view.

The objectives of this study are as follow:

1) **Determination of heavy metals in shrimp aquaculture sludge by pseudototal metal digestion**
2) **Determination and assessment of heavy metals mobility potential by Tessier method for shrimp aquaculture sludge**
3) **Determination and assessment of heavy metals mobility potential by BCR method for shrimp aquaculture sludge**

4) **Investigation of heavy metal mobility potential by modified BCR sequential extraction scheme for shrimp aquaculture sludge**
5) **Determination and assessment of heavy metals mobility potential by conventional BCR method for Sungai Buloh sediments**
6) **Comparative study of time saving between BCR sequential extraction and Tessier sequential extraction schemes**
7) **Comparative study of time saving between modified, Ultrasonic digestion and microwave assisted digestion BCR sequential extraction**

1.3- Thesis outline

The dissertation is consists of five chapters:

Chapter 1 is a general introduction about sequential extraction, including the main goals and objectives of dissertation.

Chapter 2 is the literature review and it is a general description of the soil and sediment standards used throughout the entire research. It also describes the composition of these soil and sediment standards and a review is included about conventional BCR sequential extraction schemes. Comparison between microwave assisted digestion and ultrasonic digestion method is also discussed. A review about Tessier sequential extraction procedure and identification of all sequential extraction schemes from 1970 until now are included in this chapter.

In chapter 3, both sampling sites involving shrimp aquaculture sludge and different river stations were described. Two most popular sequential extraction methods (Tessier and BCR) were described. Also all instruments employed in this work were explained. It also describes about the cleaning of reagents, glass and plastic wares used during the experimental work.

Chapter 4, discusses the results obtained in this work. Comparisons, between Tessier and BCR sequential extraction schemes in shrimp aquaculture sludge as described. Single digestion was compared with sequential extraction in both methods and contamination factor and potential mobility were measured. A modification of sequential extraction was designed and compared with conventional method.

The second sampling sites were at selected stations along Sungai Buloh and the straits of Melaka. For the latter, sediment samples were collected at different depth for each station. BCR modified sequential extraction method was used for comparative study between BCR modified sequential extraction method and microwave assisted digestion and ultrasonic digestion.

Chapter 5 presents the general conclusions made from this research.

Chapter 2:

Literature

Review

Chapter 2: Literature Review

2.1- Overview

In the late 1970s, the need to know the distribution, mobility, toxicity and phytoavailibility of heavy metals in polluted solid samples led to the organization of sequential extraction scheme (Gupta and Chen, 1975; Agemian and Chau, 1976; Stover and Sommers, 1976; Gibbs, 1977: Malo, 1977; Tessier *et al*., 1979). Recently this has been expanded to include understanding the distributions, mobility and possible uptake of radionuclides by plants (Bunzel *et al*., 1995, 1996; Rigol *et al*., 1999; Morton *et al*., 2001; Blanco *et al*., 2005; Fliegel *et al*., 2004 Fernandes *et al*., 2006; Takeda *et al*., 2006; Pett-Ridge *et al*., 2007; Galindo *et al*., 2007).

In recent years, a number of critical reviews on this topic are found (Kersten and Forstner, 1995; Ure, 1996; Kennedy *et al*., 1997; Clark *et al*., 2000; McLaughlin *et al*., 2000; Filgueiras *et al*., 2002; Quevauviller, 1998, 2002; Gleyzes *et al*., 2002; Hlavay *et al*., 2004; Young *et al*., 2006; Bacon and Davidson 2008). As a matter of fact, Kersten and Forstner (1995) reported 25 sequential extraction procedures that were applied from 1973 to 1993. There has also been a rapid increase in the different substrates on which these schemes are applied (Bacon and Davidson, 2008), including:

"a) contaminated soils (McGrath, 1996; Maiz *et al*., 1997; Lo and Yang 1998; Rauret, 1998; Berti and Cunningham, 1999; Maiz *et al*., 2000; Li *et al*., 2001; Olajire *et al*., 2003; Anawar *et al*., 2008);

b) forest soil (Ettler *et al*., 2005; Klaminder *et al*., 2005; Inaba and Takenaka, 2005; Sipos *et al*., 2005; Komarek *et al*., 2006);

c) agricultural soils (Manz *et al*., 1999; Veeresh *et al*., 2003; Koleli, 2004; Davidson *et al*., 2006; Yang *et al*., 2006; Madrid *et al*., 2007; Novoa-munoz *et al*., 2007; Li *et al*., 2007);

d) urban soils (Davidson *et al*., 2004, 2006; Banat *et al*., 2005; Peltola *et al*., 2005);

e) sewage sludge (Perez-Cid *et al*., 1999; Fuentes *et al*., 2004, Chen et al., 2005, Hullebusch *et al*., 2005; Wong and Selvam, 2006; Sanchez-martin *et al*., 2007; Fuentes *et al*., 2008);

f) composts (Ciba *et al*., 2003: Li *et al*., 2003: Szymonski *et al*., 2005: Liu *et al*., 2003, 2007);

g) airborne particulate matters (Fernandez *et al*., 2000; Dabeck-zlotorzynska *et al*., 2003, 2005, Fujiwara *et al*., 2006; Richter *et al*., 2007; Sammut *et al*., 2008);

h) medical waste fly ashes (Krishnan *et al*., 1992; Sukandar *et al*., 2006);

i) mine tillages (Clevenger, 1990; Carlsson *et al*., 2002; Mbila and Thompson, 2004; Margui *et al*., 2004; Kidd and Monterroso, 2005; Al- bed *et al*., 2006);

j) organic wastes (Chaudhuri *et al*., 2003; Bhattacharyya *et al*., 2005, 2007; Ostman *et al*., 2008);

k) coal (Finkelman, 1989, 1994, Teixeria *et al*., 1994; Laban and Atkin, 1999; Baruah *et al*., 2005; Zheng *et al*., 2008)

l) coal fly ashes (Petit and Rucandio, 1999; Smeda and Zyrnicki, 2002; Soco and Kalembkiewicz, 2007);"

Despite the wide range of sequential extraction schemes existed, only two schemes are used widely. These two schemes are the Tessier *et al*. (1979) and the Standards measurements and testing Programme (formerly BCR) of the European Community (Quevauviller, 1998a, b). Of these, the Tessier scheme is the most well-liked and has been cited more than 2500 times. According to the scheme, the labile metals are extracted as five fractions; exchangeable, carbonates (acid-soluble), iron and manganese oxides (reducible), organic matter (oxidizable) and residual fraction. The underlying hypothesis, as with all other sequential extraction procedures, is that with proper chemical reagents, the metals that are associated to different sediment phases can be displaced and extracted by increasing the strength of the reagents under controlled situation.

It follows that sequential extraction procedures depend on the reagent used and the order of extraction (Kennedy *et al*., 1997; Kim and Mcbride, 2006) and as such they are operationally defined. Gleyzes *et al*., (2002) presented a comprehensive review of more than 33 different reagents used in different sequential extractions schemes and expound on the strengths and limitations of each reagent. Amongst the different reagents, hydroxylamine hydrochloride and hydrogen peroxide are the most applied to extract metals that are bonded to manganese and iron oxides and organic matter; the two major sinks for heavy metals (Ramos *et al*., 1994; Baruah *et al*., 1996; Gleyzes *et al*., 2002; Wong *et al*., 2002). These reagents are widely used in different sequential methods, due to their reducing and oxidizing properties (Tessier *et al*., 1979; Quevauviller *et al*., 1993; Arunachalam *et al*., 1996; Lo and Yang, 1998).

Despite the fact that sequential extraction procedures are widely applied, they have come under immense criticisms for poor metal recovery, re-adsorption, redistribution and incomplete dissolution of the targeted soil fraction (Martin *et al*., 1987; shan and Chen,

1993; Bermond and Yousfi, 1997; Raksasatayal *et al*., 1996, dodd *et al*., 2000; Schramel *et al*., 2000). For instance, using a lower amount of sodium acetate in the dissolution of the "carbonate phase" in carbonate rich substrates will result in incomplete dissolution, consequently, an overestimation of lead in the next step when hydroxylamine hydrochloride is used (Gleyzes *et al*., 2002). Furthermore, the schemes are unable to extract any chemical entities other than those defined by the method itself (Nierel and Morel, 1990).

To resolve some of these problems and to tailor the Tessier procedure to meet specific needs, modifications have been made (Forstner and Salomons, 1980; Meguellati *et al*., 1983; Han and Banin, 1995; Li *et al*., 1995; Schultz *et al*., 1998; Karlfeldt and Steenari, 2007). A common feature of these improvements is a change in the order in which hydroxylamine and hydrogen peroxide extractions are done (Forstner and Salamons, 1980; Han and Banin, 1995). As such, since sequential extraction results are highly dependent on the reagents used and the order of the extraction (Kennedy *et al*., 1997; Kim and McBride, 2006), these modifications may impose other undesired consequences on the results obtained. It is difficult to judge the quality with which any sequential extraction that has been carried out, because few investigations use standards, even though many exist, making it difficult for inter laboratory comparisons and confirmation of the schemes by other researchers (Mester *et al*., 1998).

Problems such as the poor oxidizing power of hydrogen peroxide (Hall *et al*., 1996), its ability to affect metal re-adsorption (Schramel *et al*., 2000; Chomchoei *et al*., 2002), and the poor reducing power of hydroxylamine hydrochloride (Schramel *et al*., 2000) led to the proposal of a new sequential extraction scheme by Hall *et al*., (1996a, b). This scheme replaces the hydrogen peroxide used in Tessier and BCR schemes to extract heavy metals bound with "organic matter" with potassium chlorate, a supposedly stronger oxidizing

agent (Hall *et al*., 1996a) to extract heavy metals bound to "sulfides and organics". The scheme also uses stronger hydroxylamine hydrochloride solutions, 0.25 mol L^{-1} and 1 mol L^{-1}, to extract the heavy metals associated with the amorphous iron hydroxide and crystalline iron respectively, instead of the weak 0.04 mol L^{-1}hydroxylamine hydrochloride used in the Tessier scheme to extract heavy metals bound to iron and manganese oxides. Despite all these attempts and the proposal of new sequential technique, it seems the solution to the problems raised may not come from another sequential scheme because the major drawbacks to sequential extraction procedures are time, tedium and cost. For example, it takes close to 15 hours to carry out a Tessier extraction and 50 hours to carry out the original BCR procedure.

The alternative is to use a single extraction method. After all, sequential extraction method are aimed to extract a potential labile or available metal pool (Young *et al*., 2006) in whatever substrate they are applied to. Single extraction schemes are fairly simple and involve only two steps designed to extract the labile and non-labile metal pools in soils, sediments and other substrates. A critical review by Kennedy *et al*., (1997) reported 12 reagents that were used for single extractions to determine the bioavailability of heavy metals to plants. Some of the traditional reagents used are:

"a) calcium chloride (Houba *et al*., 1996; Geebelen *et al*., 2003; Pueyo *et al*., 2004);

b) chelating agents such as diethylene triamine penta-acetate (DTPA) (Qian *et al*., 1996; Fuentes *et al*., 2004; Wang *et al*., 2004; Hseu, 2006);

c) ethylene diamine tetra-acetate (EDTA) (Ure, 1996; Quevaviller *et al*., 1996; garrabrants and Kosson, 1997, 2000; sun *et al*., 2001; Perez *et al*., 2002; Amrate *et al*., 2005; Brunori *et al*., 2005). Some other reagents used in single extraction schemes are hot concentrated

nitric acid used in the EPA method 3050 (Kimbrough *et al*., 1989; Kane, 1995; Lynch, 1996; Mohamed, 1996; Binns *et al*., 2004; Finster *et al*., 2004) and cold hydrochloric acid (Sutherland, 2002, 2003; Sutherland and Tack 2008; Cook and Parker 2006)". The results from these studies showed that a labile or available metal pool which was extracted, was similar to that extracted with multiple step sequential extraction procedures. All the above authors concluded that a single step extraction provided fast, easy and cost effective method to extract the available metal pool for the purpose of environmental monitoring and determining bioavailability of metals in contaminated solid samples.

2.2-Structure and composition of soils and sediments

2.2.1- Minerals

Soil and sediment are complex mixture of solid, liquid and gas. The mineral particles which make up the principal solid phase are formed by physical and chemical weathering of the parent rock material. These particles have extensively varying sizes; those with diameters less than 2 mm comprise the fine earth fraction. The size distribution is further sub-divided into sand (> 50 µm), silt (> 2 µm), and clay particles (< 2 µm). This agglomeration is bound together by the wetness within the pore spaces between the grains, and by organic material originating from vegetation and surface litter. In nature, soil particles are usually more complex composites such as clay minerals coated with organic matter, or with inter-layered hydrous oxides of Fe and Mn. Unlike most surface soils, aquatic sediments develop in an anoxic environment. Chemical speciation in sediments will be affected on removal, requiring processing and analysis in a nitrogen atmosphere.

2.2.2- Organic Matter

Microbial action decomposed vegetation and releasing inorganic nutrients into the soil. The final products of the composition are humic and fulvic acids which are complex polymers of unspecifiable organization, with extremely high molecular weight and surface area. This material known as humus that makes up approximately 60 % - 80 % of total soil organic matter.

2.3-Heavy metals behavior in soils

2.3.1- Sources and Sinks

Inorganic soil contamination is primarily anthropogenic, as a result of fossil fuel burning, the use of pesticides, metals production and processing, and industrial manufacturing. Heavy metals also enter the environment from landfill, and from sewage sludge, which are routinely used as soil conditioners in some areas. Once in the soil, metals are generally rapidly immobilized, and will not leach out except under acidic conditions. However, this tendency also promotes accumulation of contaminants under continued loading.

The mechanisms by which metal ions bind to soil particles have been well-researched and modeled (Evans, 1989). Thermodynamic modeling of complex multi-component mixtures such as soil is usually based upon the theory that the individual phases act independently of each other, and their contributions to adsorption may simply be superposed (Tu *et al.* 1994). Four types of association are recognized. In physical adsorption, ions are weakly and reversibly bound by electrostatic forces, to oppositely charged surfaces such as clay or oxides particles. Adsorbed ions may be displaced from their surface adsorption sites by other ions, a process termed ion exchange. Specific adsorption or chemisorption involves

stronger associations, which act similarly to covalent chemical bonding. One example is the coordination of metals with surface organic ligands. Exchangeable and adsorbed metal is more readily available for plant uptake, while organic-bound metal can become available over time. The third major pathway for metals is through precipitation as insoluble secondary minerals, including carbonates, hydroxides, and sulfides. Finally, metals can be incorporated within the crystalline silicate lattice of the residual soil component, which is mostly inert.

The highest levels of pollutants are usually found in the "very fine" silt and clay fractions, because of their higher specific surface area rather than on larger grains of sand and silica. For this reason, many soil contamination tests restrict analysis to the soil fraction with particle diameters of 63 μm and less.

2.3.2- Physical adsorption and ion exchange

Ion exchange is a very rapid, surface-dependent phenomenon, mostly occurring on the surfaces of colloidal particles in soil, 2 μm or less in size. In layered silicate clays, additional internal surface area is available, greatly increasing the exchange capacity of the soil. The binding mechanism is electrostatic; soil nutrient cations are held primarily by permanent net negative charges on these surfaces. These charges result from isomorphous substitution of Si^{4+} in the silicate lattice by atoms of lower valence, such as Al^{3+} or Fe^{2+}. For any hydrated metal cation M^{n+}, ion exchange can be represented by the following equation:

$$\equiv Si\text{-}O^{-} + M(H_2O)_y^{n+} \rightleftharpoons \equiv Si\text{-}O\text{-} \ldots M(H_2O)_y^{n+} \qquad (2\text{-}1)$$

where ≡ indicates a soil solid surface, and … indicates a weak electrostatic attraction (McBride, 1994):

Ions with higher valences will be preferentially bound compared to mono-valent ions.

Under neutral or slightly alkaline soil conditions, deprotonated functional groups on the surfaces of organic matter will also become points of net negative charge, and act as cation exchange sites. Ion exchange is also strongly dependent on soil pH. With increased acidity, H^+ competes for exchange sites, and aqueous metal concentrations should be expected to increase.

Neither electrostatic nor electronegativity arguments alone can satisfactorily explain observed trends in relative adsorption affinities for cations of equal charge. It has been suggested that affinity is proportional to ionic radius, and therefore inversely proportional to ionic potential, which is the ratio of charge to ionic radius. Ions with low ionic potential are less likely to remain hydrated, promoting interaction with solid surfaces. Larger ions are also more polarizable, which permits the formation of stronger covalent bonds.

2.3.3- Chemisorptions and surface complexation

While nutrient cations must be exchangeable, most divalent heavy metal cations will be chemisorbed, and much less removable. Surface complexation to phenolic and carboxylic ligand groups leads to the formation of both hydrated outer sphere, and highly stable inner sphere complexes with organic material. Metal complex stability constants for these ligands increase in the order Zn < Co = Mn < Al = Fe < Cu (Alloway, 1995).

The Irving-Williams series of divalent metal-organic affinities runs:

Ba < Sr < Ca < Mg < Mn < Fe < Co < Ni < Cu < Zn (McBride, 1994).

Hydroxyl groups of hydrous oxides of iron, manganese, and aluminum will also form very strong complexes (Mcbride, 1994):

$$\equiv Fe\text{-}OH^{-1/2} + M(H_2O)_y^{n+} \rightleftharpoons \equiv Fe\text{-}O\text{-}M(H_2O)_{y-1}^{(n-3/2)} + H^+ \quad (2\text{-}2)$$

Chemisorptions are also pH-dependent, as pH is the master variable controlling complexation equilibria. The stability constants of metal-hydroxy complexes increase in the order: Cd < Ni < Co < Zn << Cu < Pb < Hg (Alloway, 1995). Oxides of Mn have particularly high affinity for Cu, Ni, Co and Pb, while Fe oxides strongly adsorb Pb and Cu (McBride, 1994).

2.3.4- Heavy metals in the natural environment

Heavy metals occur naturally in soils (**Table 2.1**); it is only when concentrations are significantly elevated or toxic that their presence becomes a concern. Metals are especially hazardous when they are mobile, and can enter the food chain, or be transported over long distances. Despite variations in experimental results, general agreement in the literature is found on behavioural trends and tendencies for the metals of interest. With knowledge of the soil chemistry and adsorption kinetics, it is possible to make predictions about the mobility, and the preferred sinks of each particular metal. For example, Cd is known to be highly mobile, while other metals, particularly Cr and Ni, are more strongly retained. Both Cu and Pb have very high affinity for organic matter.

Relative adsorption selectivity for different metals is largely described by the Lewis hard-soft acid-base (HSAB) principle (Alloway, 1995). Complexation is favored between acid and base pairs of equal hardness, where “hard” implies high electronegativity, low polarizability, and small ionic radius (**Table 2.2**). Hard acids target O-containing ligands, and form hydrated outer sphere complexes. Soft acids prefer S, P or N-containing ligands, and form inner sphere complexes, resembling covalent or coordination bonds (Logan *et al*., 1997).

Table 2.1 – Soil chemistry of selected heavy metals

Element	Background Soil Concentrations	Major Sink(s) (in decreasing significance)
Cd (Alloway, 1995)	0.1 – 1.0 µg g^{-1}	- more mobile than other metals - weakly adsorbed or in solution as Cd^{2+} or complexes of Cl^- and OH^-
Cu (Baker and Senft, 1995)	20 – 30 µg g^{-1}	organic matter, Fe/Mn oxides, clays
Pb (Davies, 1995)	30 – 100 µg g^{-1}	organic matter, Fe/Mn oxides
Zn (Kiekens, 1995)	10 – 300 µg g^{-1}	strongly chemisorbed to clays and organic matter, Fe/Mn oxides
Cr (McGrath, 1995)	30 – 80 µg g^{-1}	-insoluble Cr oxides and hydroxides -complexed to organic matter
Ni (McGrath, 1995)	20 – 40 µg g^{-1}	-residual fraction, Fe/Mn oxides -low exchange affinity
U (Lepp *et al.*, 1995)	1 – 10 µg g^{-1}	mobile in oxidizing conditions
As (kabata-Pendias and Pendias, 1992)	4 – 30 µg g^{-1}	-dependent on oxidation state -strongly bound to Fe oxides

Table 2.2 – Lewis HSAB properties

Type	Acid	Base
Hard	Na^+, K^+, Mg^{2+}, Ti^{4+}, Cr^{3+}, Mn^{2+}, Fe^{3+}	Water Fe oxides O-functional groups
Soft	Cu^+, As^+, Cd^{2+}, Hg^+ and Hg^{2+}	clay minerals S, P or N-functional groups
Intermediate	Fe^{2+}, Ni^{2+}, Zn^{2+}, Sb^{3+}, Pb^{4+}	

2.4-Soil sampling and preparation

2.4.1- Soil sampling

In any analysis of soils, the field sampling methodology is usually the weakest link (Brady and Weil, 1996). Due to the great heterogeneity of soil, both laterally and in depth, it is often difficult to know whether a sample is truly representative of the large area. External influences such as seasonal effects may also need to be accounted for. Typically, a soil probe or auger is used to remove thin cylindrical cores from 15 to 20 randomly scattered points within a sampling area, avoiding obviously unusual soil features or formations. These samples are combined and thoroughly mixed to produce a composite sample for testing.

2.4.2- Soil preparation

Since field moisture levels are neither constant, nor comparable from soil to soil, collected samples should be dried before analysis. Drying can affect N and P analysis, drive off volatile elements, and even change chemical speciation. However, as long as temperatures are kept below 40 ºC, soil drying is recommended for convenience. Dried samples should remain stable in low humidity storage for months or even years (Bates, 1993). For strictly elemental analyses of the soil, drying and storage should not be a major factor.

Sediments, especially in anoxic zones, are more easily affected by sample handling and preparation. Extractability of several metals and sulphides are affected by freeze-drying and oven drying (Rapin *et al.,* 1986). Sterilization by γ-irradiation will stop microbial action, and increases the sample stability over time.

Soil tests are usually performed on the fine earth fraction which passes through a 2 mm nylon or stainless steel screen. Any grinding or milling action used should be sufficient to break up aggregations, but not the individual practices. Sample contamination from metal and sieves is generally negligible (Bates, 1993). By removing larger practices, the minimum mass of soil comprising a representative sample size is greatly reduced. This representative sample should contain at least 1000 grains, but preferably several times this number. This requirement is less important for highly contaminated soils (Rubio and Ure, 1993).

Particle size separation is usually accomplished by sieving through a series of meshes, although some workers prefer gravity sedimentation or differential centrifugation in a large vessel. The main objective is to minimize distortion in results due to very fine material existing as aggregates (Pickering, 1995). This grain size effect can cause variation greater than one order of magnitude (Kersten and Forstner, 1995).

Although contaminations will tend to be concentrated in the finer fractions, 90 % or more of the mass in some soils and sediments can be sand size, and even low concentrations in this fraction will still represent a major sink (Jain and Ram, 1997). In polluted soils, contaminant metals may be in the form of particulates of the pure metal, in which case soil particle sizes will have little bearing on metal concentrations. The coarse sand fraction will appear for significant proportions of the contamination (Yarlagadda *et al*., 1995).

2.5 Analytical Techniques

2.5.1- Atomic Absorption Spectrometry (AAS)

Heavy metals analysis was largely driven by the development of flame atomic absorption spectrometry (FAAS). A hollow cathode source lamp made of the analyte metal is used to produce a monochromatic beam of atomic emission radiation, which is passed through a sample atomized in a chemical flame. The beam is characteristic to the element of interest, so that only atoms of that element will absorb radiation at this wavelength. The attention of the beam after passing through the sample is proportional to the number of analyte atoms in the sample, and can be calibrated to determine concentrations. Although electrothermal (ETAAS) atomization techniques such as graphite furnace (GFAAS) atomization have the best analytical capabilities available for some elements, they are far more complicated and expensive than FAAS.

Non-spectral or chemical interferences can also be a significant problem in AAS. For example, Pb will form acetate complexes which will not absorb at the same wavelength as the metal atom, suppressing the measured Pb concentration (Varian Manual, 1993). This may pose a problem with sample matrices of acetic acid or ammonium acetate. The linearity between absorbance and atomic concentration holds only for dilute solutions, typically less than 2 ppm. Samples to be analyzed must fall within this range, and soil extracts usually require significant dilution. Outside of a few recently developed instruments, AAS remains a single element technique. Although simple to use and widely available, AAS has largely been supplanted by atomic emission spectrometry techniques.

2.5.2- Atomic Emission Spectrometry (AES)

Unlike AAS, which measures the absorption of optical radiation by the sample, AES measures the radiation emitted at the same wavelength by the sample atoms under excitation conditions. It had limited usage before the advent of the inductively coupled plasma (ICP). Powered by electromagnetic forces, the temperature within argon gas plasma can reach temperatures up to 10000 K, ensuring complete sample atomization. Since only argon is used in the excitation source, interfering background emissions are low. Moreover, the proportionality of emission intensity to concentration extends over five or more orders of magnitude, often up to 10000 ppm. With this large dynamic operating range and modern optical spectrometers which can simultaneously filter out thousands of wavelengths with high resolution, ICP-AES easily analyses 30 or more elements at once. Environment analysis applications now routinely use ICP-AES.

Coupling the ICP with mass spectrometry (ICP-MS) yields far superior detection limits, and also permits isotopic ratio analysis, which can be used for identification of contamination sources (Dean *et al.,* 1997).

2.6- Sequential Extraction Procedures for Sediment Analysis

2.6.1- Introduction

Sediments play an important role in aquatic systems both as a sink where contaminants can be stored and as a source of these contaminants to the overlying water and to biota. Due to their ability to sequester metals, sediments are a good indicator of water quality and record the effects of anthropogenic emissions (Baudo *et al*., 1990). Thus, sediments are widely used in environmental studies.

Biogeochemical and especially the ecotoxicological significance of a pollutant is determined by its specific binding form and coupled reactivity rather than by its accumulation rate in sediments and due to this fact, the sediment analysis techniques for evaluation of environmental impact of polluted sediments has changed from the determination of total concentrations towards a more sophisticated fractionation of the sediment compounds (Kersten *et al*., 1989).

Trace metals in sediments may exist in different chemical forms or ways of binding. In unpolluted soils or sediments trace metals are mainly bound to silicates and primary minerals, relatively immobile species, whereas in polluted ones trace metals are generally more mobile and bound to other sediment phases (Rauret, 1998). However, the measurement or evaluation of this speciation, way of binding or fractionation is very difficult due to the intrinsic complexity of the sediment water biota system. Different variables determine the behaviour of these systems: the genesis of the sediment (Forstner, 1993), the type of weathering products and the processes which control the transport and redistribution of the elements (adsorption, desorption, precipitation, solubilisation,

flocculation, surface complex formation, peptisation, ion exchange, penetration of the crystal structure of minerals, biological mobilization and immobilization) (Plant *et al*., 1983). Almost all the problems associated with understanding the release processes that control the availability of trace metals concern particle water interfaces.

There are a variety of well established methods to assess the environmental impact of a selected contaminant in sediment water systems which range from pore water gradient measurements, in situ or laboratory incubation experiments up to leaching approaches such as sequential extraction procedures. While the first two approaches aim to study the actual trace metal release potential, the latter approach is widely applied as a predicting tool for the long-term emission potential of sediments (Sloot, *et al*., 1997).

Different sequential extraction schemes have been proposed for the determination of binding forms of trace metals in sediments. These schemes are a good compromise that provides a practical method for giving information on environmental contamination risks in spite of being operationally defined procedures.

2.6.1.1- Commonly Used Sequential Extraction Procedures for Sediments

The phases considered relevant in heavy metal adsorption in sediments are oxides, sulphides and organic matter. The fractions obtained when applying sequential extraction schemes are related to exchangeable metals, metals mainly bound to carbonates, metals released in reducible conditions such as those bound to hydrous oxides of Fe and Mn, metals bound to oxidizable components such as organic matter and sulphides, and a residual fraction. **Table 2.3** summarizes the extractants most commonly used to isolate each fraction (Ure, *et al*., 1993;). Some experimental conditions have been included in **Table 2.3**

but there are many variations described involving pH and temperature of the reaction medium, presence of chelating and buffering agents, concentration of the reagent, *etc*.

Although the extractants shown in **Table 2.3** are selective, few of them are specific enough to isolate well-defined fractions and the different fractions of the sediment phases appear overlapped. The fractions can, however, be isolated prescribed order.

From the sequential extraction schemes described in the literature, the classical method of Tessier (Tessier, *et al*., 1979) has been widely applied in river, marine and stream sediments (Lopez-Sanchez, *et al*., 1996), in municipal composts and sewage sludges (Zufiaurre, *et al*., 1998;), and also in soils (Li, *et al*., 1995; Maiz, *et al*., 2000). Several modifications to the Tessier scheme have been proposed by different authors in order to improve the selectivity of the extractants agents towards specific geochemical phases of the sediment. **Table 2.4** shows a scheme of the Tessier sequential extraction procedure together with two modifications made by Forstner (1993) towards a more specific isolation of the Fe and Mn oxide and hydroxide phases and by Megullati (1983) who isolates the organically bound phase before the carbonate-bound.

Other proposed modifications which deal with the possibility of accelerating the extraction procedure by the application of ultrasound (Perez-Cid *et al*., 1999) or with the use of different extractant agents to avoid the readsorption problems observed in oxic sediments (Gomez-Ariaz, *et al*., 1999).

In addition to the elaborate sequential extraction schemes, there are a number of simpler procedures with a smaller number of steps (Forstner et al., 1980; Bafi, *et al*., 1992; Cosma, *et al*., 1991) which are summarized in **Table 2.5** While the more elaborates schemes are more useful and informative in terms of the physical chemistry of systems and for an

understanding of the mechanisms of immobilization, release and transport, the simpler schemes may well be quite sufficient for practical assessment of the extent of pollution of sediment and the potential dangers of its use in agriculture, landfill, etc. Moreover optimization studies are recommended to increase the repeatability when using the longer schemes (Accomasso, *et al*., 1993).

The results obtained from the sequential extraction schemes are operationally defined and different mineralogical compositions and organic matter content lead to different efficiencies of extraction and readsorption. An individual method validation is required for each matrix (Nirel, *et al*., 1990) when the aim of the study is to completely isolate sediment fractions.

Table 2.3: commonly used extractants and associated sediment phases

Metal fractions	Type of extractant	Extractant used
Water soluble fraction	Water (distilled or deionised)	Pore water or H_2O extraction
Exchangeable and weakly adsorbed fraction	Salts of strong acids and bases or salts of weak acids	KNO_3 or $Mg(NO_3)_2$ $CaCl_2$ 0.01-0.05 mol L^{-1} $MgCl_2$ 1 mol L^{-1} (pH=7) $BaCl_2$ 1 mol L^{-1} (pH=7) NH_4CH_3COO 1mol L^{-1} (pH=7 or 8.2) $NaCH_3COO$
Carbonate bound fraction	Acid or buffer solutions	CH_3COOH 25% or 1 mol L^{-1} $NaCH_3COO$ 1 mol L^{-1}/CH_3COOH (pH=5) HCl EDTA 0.2 mol L^{-1} (pH=10-12)
Fractions bound to hydrous oxides of Fe and Mn	Reducing solutions other agents	NH_4CH_3COO 1 mol L^{-1} + 0.2% hydroquinone $NH_2OH.HCl$ 0.02-1 mol L^{-1} in CH_3COOH or HNO_3 $(NH_4)_2C_2O_8$ $(NH_4)_2C_2O_8$ 0.2 mol L^{-1}/$H_2C_2O_8$ 0.2 mol L^{-1} in ascorbic acid 0.1 mol L^{-1} $Na_2S_2O_4$/Na-citrate/citric acid $Na_2S_2O_4$/Na-citrate/$NaHCO_3$ (DCB) H_2O_2 10% in 0.0001 N HNO_3 HCl 20% EDTA 0.02-0.1 mol L^{-1} (pH=8-10.5) Hydrazine chloride (pH=4.5)
Organically bound and sulphidic phase	Oxidizing reagents	H_2O_2 in HNO_3 + extraction with NH_4CH_3COO or $MgCl_2$ NaClO (pH=9.5) Alkali pyrophosphate ($Na_4P_2O_7$ or $K_4P_2O_7$) H_2O_2/ascorbic acid HNO_3/tartaric acid $KClO_3$/HCl
Residual fraction	Strong acids	Alkaline fusion HF/$HCLO_4$/HNO_3 Aqua regia HNO_3/H_2O_2 HCl/HF/HNO_3

Table2.4: Tessier's scheme and proposed modification

Sequential extraction procedure	Step1	Step2	Step3	Step4	Step5	Step6	Step7
Tessier (Tessier et al. 1979)	Exchangeable $MgCl_2$ 1mol L^{-1} pH=7		Carbonatic NaOAc 1 mol L^{-1}/ HOAc pH=5	Oxides Fe/Mn $NH_2OH.HCl$ 0.04 mol L^{-1} in 25% HOAc		Organic matter and sulfidic H_2O_2 8.8 mol L^{-1}/ HNO_3 and NH_4OAc 0.8 mol L^{-1}	Residual $HF/HClO_4$
Forstner (Forstner et al. 1993)	Exchangeable NH_4OAc 1 mol L^{-1} pH=7		Carbonatic NaOAc 1 mol L^{-1}/ HOAc pH=5	Easily reducible $NH_2OH.HCl$ 0.1 mol L^{-1}	Moderated reducible 0.1 mol L^{-1} oxalate buffer	Organically matter and sulfidic H_2O_2 8.8 mol L^{-1}/ HNO_3 and NH_4OAc 0.8 mol L^{-1}	Residual HNO_3
Meguellati (Meguellati et al. 1983)	Exchangeable $BaCl_2$ 1 mol L^{-1} pH=7	Organic matter and sulfidic H_2O_2 8.8 mol L^{-1} (HNO_3) and NH_4OAc 0.8 mol L^{-1}	Carbonatic NaOAc 1 mol L^{-1} HOAc pH=5	Oxides Fe/Mn $NH_2OH.HCl$ 0.04 mol L^{-1} in 25% HOAc			Residual Ashing and HF/HCl

Table 2.5: **Short Sequential extraction Scheme**

Sequential extraction procedure	**Step1**	**Step2**	**Step3**	**Step4**
Forstner (Forstner et al. 1993)	Exchangeable and carbonatic NH2OH.HCl pH=2		Organic matter and sulfiddic H_2O_2 8.8 mol L^{-1}/ HCl and NH_4OAc 0.8 mol L^{-1}	Residual $HF/HClO_4$
Banfi (Banfi et al. 1992)	Exchangeable NaOAc 1 mol L^{-1}/ HOAc pH=5	Oxides Fe/Mn $(NH_4)_2C_2O_8$ 0.2 mol L^{-1}/$H_2C_2O_8$ 0.2 mol L^{-1} pH=2	Organic matter and sulfiddic H_2O_2 8.8 mol L^{-1}/ HCl and NH_4OAc 0.8 mol L^{-1}	Residual Aqua regia
Cosma (Cosmo et al. 1991)	Exchangeable NH4OAc 1 mol L^{-1}	Oxides Fe/Mn NH2OH.HCl 1 mol L^{-1} in 25%HOAc	Organic matter and sulphiddic HNO_3	

2.6.2- Preliminary studies of BCR

The main problem of sequential extraction appears when the results obtained from different procedures are compared because the data, as stated before, depend on experimental conditions (Lopez-Sanchez, *et al*., 1993). Moreover, the lack of suitable reference materials for this type of analysis does not enable the quality measurements to be controlled. Results are useful and usable only if they correspond to well-defined and accepted procedures and with assessed accuracy to make the results comparable elsewhere (Girepink, *et al*., 1993; Quevauviller, 1998).

The BCR method began in 1987 as a series of investigations and collaborative studies with the aim of harmonizing and improving the methodology for sequential extraction determination of trace elements in sediments. The information obtained with an initial study of literature on the topic (Ure, *et al*., 1992) was discussed by a group of representatives of leading European soil and sediment laboratories and as a conclusion it was considered that the organization of an interlaboratory trial for testing different sequential extraction schemes was essential as a first step towards the harmonization of schemes and the preparation of certified reference materials for Cd, Cr, Cu, Ni, Pb and Zn (Ure, *et al*., 1993).

Different sequential extraction schemes were tested by four laboratories on seven sediments. The procedures tested were the modified Tessier procedure by Forstner and Salomons with three steps (**Table 2.5**). The results obtained showed that most of the metals were extracted in the first step in the most mobile phases and the agreement in the

obtained information of the schemes was better for highly polluted sediments with extractable metal amounts high enough to avoid analytical problems in the determination.

It was shown that all the tested procedures would be able to classify the predominant nature of the sediments and allow recommendations to be made for their use. Thus, it would be possible to define a simple sequential extraction scheme for characterization of the sediments that was practical and selective enough for managerial decision on their use and it was recommended that a reference material be prepared according to the defined scheme for quality control purpose.

One aspect to be checked when considering the possibility of preparing reference materials for extractable metal contents is the stability of the solid. A study carried out by Salomons et al (1987) using a sequential extraction procedure with five steps showed that the conclusions drawn on five sediments after an interval of 12 years were similar to management decisions on their use, showing the stability of the extracted fractions.

2.6.3- Proposal for a common Three-step sequential extraction scheme

The first step for the adoption of a common sequential extraction scheme recommended in the previous project was the organization of a workshop. The aim of the workshop was to establish the state of the art of extractable trace metal determination, to investigate where limitation existed and discuss the work necessary to overcome these limitations as well as identified sources of error. The workshop was held in Sitges (Spain) in1992, in the framework of the project ETMESS (Extractable Trace Metal contents in Soil and Sediment) and a three step sequential extraction procedure was proposed by a group of European experts.

The scheme was tested in two round robin exercises with the aim of validating the procedure and demonstrating the feasibility of the preparation of materials that the basic requirement of a CRM for stability and homogeneity not as regards the total metal content but for the sequentially extractable metal content. Two river sediments were selected for the intercomparison exercises with 20 laboratories. The characteristics of the selected materials are shown are in **Table 2.6**.

Many systematic errors were found to be related to calibration errors in the first intercomparison. The reproducibility obtained in the extracts in terms of CVs was quite poor, especially for Ni and Pb. The effects of type of shaking, speed and temperature were checked in the second exercise. It showed an improvement in comparison with the results of the first exercise with CV less than 20% for Cd and Ni in all steps. The discussion of the obtained results in a technical meeting allowed operational details to be added to the protocol that were considered useful from a practical point of view in order

to improve the reproducibility among the laboratories. These operational details were: the speed of shaking; special care when adding the oxidant agents in sediments with high organic matter content and; recommendations for the measuring step (Fiedler, 1995; Quevauviller, *et al*., 1994). The results of the homogeneity and stability studies carried out on the sediment used in the second exercise showed that it would be possible to prepare a stable sediment candidate reference material to be certified for the extractable contents by applying the common sequential extraction procedure proposed by the BCR (Fiedler, *et al*., 1994). Thus, the first certification campaign was undertaken. The sediment candidate was lake sediment collected from different sampling sites of Lake Maggiore (Italy) (see **Table 2.6**) and it was prepared according to the same scheme used for preparing the material previously used.

Table 2.6: Reference and certified reference materials using the European three-step sequential

Sediment sample	Origin	Characteristics	Sequential extraction scheme applied	Sieving
1st interlaboratory trial (River sediment)	Yrseke (The Netherlands)	Siliceous	Original BCR	< 90 µm
2nd interlaboratory trial (River sediment)	River Besos (Barcelona, Spain)	Calcareous	Original BCR	< 63 µm
BCR CRM 601 Lake sediment	Lake Maggiore (Varese, Italy)	Siliceous	Original BCR(certified values) Modified BCR (informative values)	< 90 µm
BCR CRM 701 Lake sediment	Lake Orta (Piemonte, Italy)	Siliceous	Modified BCR(certified values)	< 90 µm

The homogeneity and stability studies were carried out with this new sediment material corroborated the feasibility studies about the possibility of certification of extractable trace metal amounts following a standardized sequential three step extraction procedure (Quevauviller, *et al*., 1997). Extractable contents of Cd, Cr, Ni, Pb, and Zn were certified in the first step of the procedure, Cd, Ni and Zn in the second step and Cd, Ni and Pb in the third step (Quevauviller, *et al*., 1997). Cu in the first step and Pb in the second step were given as indicative values. The rest of the metals were not certified due to the wide spread of the obtained results or owing to a suspicion of instability. The BCR CRM 601 constituted the first available tool for the validation of methodology in the sequential extraction research field.

Since its proposal in 1993 the three step sequential extraction scheme of the BCR has been widely applied to different type of sediments (Davidson, *et al*., 1994; Sahuquillo, *et al*., 1995; Belazi, *et al*., 1995; Marin, *et al*., 1997; Mester, *et al*., 1998; Martin, *et al*., 1998; Userno, *et al*., 1998; Tokalioglu, *et al*., 2000), contaminated soils (Raksasataya*, et al*., 1996; Maiz, *et al*., 1997; Ho, *et al*., 1997), industrially contaminated made up ground (Davidson, *et al*., 1998), sewage sludge (Perez-Cid, *et al*., 1996) and fly ashes (Petit, *et al*., 1999). The scheme was found to be sufficiently repeatable and reproducible for fresh water sediments although it was pointed out the necessity of further investigations to identify the factors responsible for variability between replicate measurements (Davidson, *et al*., 1994). Smaller variabilities were also found in its application to marine reference sediment but long term precision for some elements in some of the extracts was higher than 15%.

As far as the phase selectivity is concerned, the geochemical phase specificity has been shown to be of varying quality as determined upon single substrates. Calcium carbonate and manganese oxides released most bound metal into the expected reagents (acetic acid

and hydroxyl ammonium hydrochloride, respectively), while organic matter as humic acid generally released metal earlier in the procedure than might be expected (Whalley, *et al*., 1994; Cooetze, *et al*., 1995). The operational nature of the scheme has again been pointed out and several repetitive extractions (up to a total of eight) must be carried out in steps two and three to completely release the metal bound to iron oxides and organic matter, respectively, in metal polluted sediments (Gomez-Ariaz, *et al*., 2000).

Different conclusion can be drawn when comparing the BCR scheme with other widely used schemes according to the literature. A higher metal release, especially under reducing conditions, was shown using the modified Tessier scheme if compared to both the BCR and Maguellati procedures. These two methods later presented comparative results for the reducible and residual phases. Significant Hg losses were found using the BCR procedure but the quantification of the acetic acid phase for Cd, Cr and Ni was more reliable than that obtained with the modified Tessier and Meguellati schemes (Gomez-Ariaz, *et al*., 2000).

Although Tessier and BCR sequential extraction schemes were postulated for sediments, they have been also applied to soils. With these materials, a significant correlation was obtained between metals extracted from different types of soils regarding both metal distribution except for Pb (Ho, *et al*., 1997) and the evaluation of plant availability of metals (Zhang, *et al*., 1998).

The BCR procedure applied to sediments with a high enough total metal concentration was strongly recommended instead of longer and more difficult procedures for quantifying the fraction of metal characterized by the highest mobility and availability (Mester, *et al*., 1998).

In an attempt to simplify the methodology, a recent study compared the results obtained by two sequential extraction procedures (Tessier and BCR scheme) with those estimated

from single extractions using identical operating conditions applied in each individual fraction. The test was performed in sewage sludge for Cu, Cr, Ni, Pb and Zn extractable contents. Although for some metals the information obtained was basically the same, the use of single extractions might be only useful for a fast screening of the possible mobility and bioavailability of metals in the environment (Fernandez, *et al*., 2000).

Thus, although some amendments could be proposed to the BCR scheme in order to improve both reproducibility and phase selectivity, the European scheme is a valuable tool for predictinity potential remobilization of metals and it has proved to be a good compromise between the information obtained and the practicality in its application in the laboratory.

2.6.4 Improvement of the BCR Sequential Extraction Scheme

The difficulties reported when using the BCR sequential extraction scheme relating to the irreproducibility shown in the first certification campaign highlighted the need for refinement of the procedure. Thus, the project "Trace Metal Extraction from Sediments and Soils (TRAMES)" was undertaken in the framework of the SM&T programme (follow-up of the BCR programme).

A systematic study to assess the sources of uncertainty was carried out focusing mainly on the second step of the sequential extraction procedure and using the lake sediment BCR CRM 601 as the test sample (Sahuquillo, *et al*., 1999).

The variables and ranges tested are shown in **Table 2.7**. Of the variables considered, the pH of $NH_2OH.HCl$ in the second step proved to be the most relevant, especially for Cr, Cu and Pb extraction which showed a dramatic decrease in both extractability and reproducibility as pH increased. The rest of the studied factors did not show significant effect upon reproducibility. From this systematic study the proposed modifications to the scheme were: the use of 0.5 mol L^{-1} $NH_2OH.HCl$ adjusted to pH 1.5 by the addition of a fixed volume of dilute HNO_3 to the extractant solution and for all steps the speed of centrifugation was increased from 1500 to 3000 g.

The combination, in the modified scheme, of an increase in the concentration of $NH_2OH.HCl$ from 0.1 to 0.5 mol L^{-1} with a pH lowered to 1.5 also provided a better release of metals bound to hydrous oxides of manganese.

A small-scale interlaboratory study with eight participating laboratories was undertaken with the aim of comparing the original and modified protocols using the BCR CRM 601 (Rauret, *et al*, 1999). As a significant improvement concerning between laboratory reproducibility was observed for all metals in the step 2 and similar to smaller

uncertainties were obtained for steps 1 and 3, it was agreed to adopt the modified protocol for the preparation of new certified reference sediment.

The data from this interlaboratory exercise confirmed the stability of extractable contents of Cd, Cr, Ni, Pb and Zn in the existing CRM (Lopea-Sanchez, *et al*., 1998) according to the original BCR sequential extraction scheme and gave informative values for the extractable amounts following the modified BCR scheme.

2.6.4.1- New Sediment Certified Reference Material for Extractable Metal Contents

A candidate sediment reference material to be certified according to the modified BCR-scheme was collected from Lake Orta (Piemonte, Italy) and it was prepared in the Environment Institute of the Joint Research Centre in Ispra (Italy) following the validated procedure used for the preparation of CRM 601. **Table 2.7** shows some characteristics of the sample. The material was certified (BCR CRM 701) in an intercertification campaign for extractable contents of Cd, Cu, Cr, Ni, Pb and Zn in the three steps and it has been available for purchase from the Institute for Reference Materials and Measurements (IRMM) since the beginning of 2001 (Rauret, *et al*., 2001). The studies of homogeneity and stability of the material as well as the certification campaign have been published (Pueyo, *et al*., 2001).

Table 2-7: Variables studied as sources of uncertainly in the BCR procedure

Variable	Studied range or parameter	Investigated step
pH of extracting agent	1.0-3.0	Step 2
Type of acid used for adjustment	HCl or HNO_3	Step 2
Extraction temperature	20, 26, 40ºC	Step 2 and 3
Extraction time	2-24 h	Step 2
Inert atmosphere	N_2	Step 2
Liquid/solid phase separation	Filtration Speed of centrifugation Time of centrifugation $MgCl_2$ as washing solution	Step 2 and 3
Extractant concentration	0.1-1 mol L^{-1}	Step 2
Alternative reagents	$NH_4HC_2O_8$ $H_2C_2O_8$	Step 2

2.6.4.2- Recent Applications of the modified BCR Sequential Extraction Scheme

The amended protocol has been recently applied to Cd, Cu, Cr, Ni, Pb and Zn determination in stream sediments from Lake Flumendosa (Italy) (Sahuquillo, *et al*., 2000). The basin of Lake Flumendosa has been mined extensively leaving behind large dumps of mining wastes. Residual metals are mobilized from these wastes and accumulated in the sediments of the lake, known to show the highest Cd concentrations ever encountered in European sediments (Cireddu, *et al*., 1997). The conclusions of this study showed that this procedure can be applied to field samples from different origin, composition and within a wide range of total metal contents with high reproducibility in all steps (most values around 5-6 % RSD for triplicates).

Another lake ecosystem (Lake Montorfano, Como, Italy) has also been studied applying the modified scheme to assess the potential remobilization of Cd, Pb, and Zn which were present in high concentrations. The sediments of this lake had a high organic matter content (from 16 to 49 %) which made them extremely reactive at the initial addition of the oxidant reagent used in step 3 and could present serious difficulties (Sahuquillo, *et al*., 1995). The modified protocol has proved to be reproducible even with these high organic matter contents by paying special attention to the digestion step with H_2O_2 (Serano, *et al*., 2000).

2.7- Certification of Soil Reference Materials

2.7.1- Preparation of the Reference Materials

2.7.1.1- CRM 483

The material was collected from Great Billings Sewage farm (Northampton) in 1991 (Quevavuviller, *et al*., 1997). Some 300 kg of field moist soil was collected by multiple sampling to a depth of 10 cm and bulked into polyethylene bags for transport to the Macaulay Land Use Research Institute (Abedeen, UK). The whole soil was air dried at 30 ºC for 3 weeks on paper lined aluminum trays. The dried material was then gently rolled with a wooden roller to break up large aggregates, sieved through a 2 mm round-hole sieve and stored in tightly sealed polyethylene bags. The soil sample was thoroughly mixed and homogenized by rolling on a clean polyethylene sheet for three days with occasional- mixing by hand. The whole sample was then gently poured on to a clean polyethylene sheet, mixed and coned and quartered by hand. The initial sample, nominally 150 kg of air dry (< 2mm) soil was split by coning and quartering, bulking opposite quarters to form the half samples, and setting one half sample aside. The remaining half sample was again coned and quartered. The coning and quartering procedure continued (six cycles) until the half sample weight was approximately 2 kg. From opposite quarters of this half sample 20 subsamples were taken alternately by nylon spatula into precleaned brown glass bottles (capped by polyethylene screwcaps). Each bottle contained approx. 70 g and a total of 1280 bottles were obtained. 128 bottles (two from each final half sample) were set aside for homogeneity and stability testing.

2.7.1.2- CRM 484

The sampling of the material (terra rossa soil) was carried out in 1991 in a farm plot amended with sewage sludge from a water treatment centre located in Northeast Catalonia, Spain (Quevavuviller, *et al*., 1997). Samples were taken from an area of about 250 m^2 with a small shovel to a depth of about 10 cm and sifted on site by hand through a 0.5 cm nylon sieve into polyethylene bags. The samples were taken to the water Treatment Centre and again sieved through a 20 cm diameter nylon sieve with a mesh size of 2 mm into polyethylene bags for transport to the laboratory of Analytical Chemistry of the University of Barcelona. The soil was then spread over a polyethylene sheet and air dried at 30ºC for one week to final water content of 1.5 %. The air dried soil was packed into a 100 liter polyethylene container, tightly sealed and dispatched to the Environment Institute of the Joint Research Centre of Ispra (Italy) for homogenization and bottling. The air dried (< 2 mm) soil sample was transferred in total (91 kg) into a mixing drum filled with dry argon and placed on a roll bed capable of handling 100 kg samples. The homogenization of this soil, with its large spread of particle sizes, from just below 2 mm down to fractions of a micrometer, required particular care. This procedure was, therefore, carried out by mixing in the drum for over 4 weeks. The bottling procedure was performed as follows: to prevent segregation of fine particles, 10 samples were taken from the centre of the drum immediately upon stopping the rotation of the mixing drum, and were placed into 10 pre-cleaned brown glass bottles, so each contained minimum of 70 g of soil. The drum was again rotated for a further two minutes and a further 10 samples were sub-sampled in the same way into bottles. The sub-sampling and bottling operation was continued until 1000 bottles of the soil were obtained. 100 bottles, selected sequentially over the whole bottling procedure were sent to the Macaulay Land Use Research Institute for homogeneity and stability testing.

2.7.1.3- CRM 600

The material was collected at San Pellegrino (Italy) in February 1994, following a prospective study of various sites in Italy, which aimed at identifying a material with reasonably high calcium carbonate content (Quevavuviller, *et al*., 1996; 1997). About 250 kg of soil was collected by shovelling from the surface to a depth of 10 cm (collection of top layers). Stones and large plant litter were removed prior to sieving with a 2 mm mesh. The fraction less than 2 mm was collected in stainless steel trays in which the material was dispersed in thin layers of a few cm of thickness to dry at ambient temperature. The material was sieved again after drying to remove lumps which formed during the drying process. The residual moisture content at this stage was found to be 3.8% (measured by taking a separate portion of 1 g dried at 105 ºC until constant mass was attained). The sieved material was transferred into PVC mixing drum filled with dry argon, and was homogenized for 12 days at about 48 rpm. The final material was manually bottled in brown glass bottles. The bottling procedure was carried out by filling 10 bottles, closing the drum and mixing the material again for 2 min before bottling another 10 bottles, and so on until only a few cm of soil remained in the drum (which was discarded). All bottles were closed with an insert and a screw cap and stored at ambient temperature. 1050 bottles each containing about 70 g was produced.

2.7.1.4- BCR 700

The material is an organic rich soil which was selected from the bank of Euro-soils of the Joint Research Centre of Ispra (Rauret, *et al*., 2001; Pueyo, *et al*., 2001). The sampling of the material was carried out in 1998 at Hagen, Germany. Samples were taken from an area of about 200 m^2 by the Environmental Institute of the Joint Research Centre of Ispra (Italy) for homogenization and bottling. The collected material was picked over for the removal of stones, litter and other material extraneous to soil and exposed to air drying at ambient temperature. Following air drying, the soil aggregates were crushed and the soil passed over a 2 mm sieve. The fraction > 2 mm was discarded. The soil fraction (91 kg) was transferred into a mixing drum filled with dry argon and placed on a roll bed capable of handling 100 kg samples. The homogenization of this soil, showing a large spread of particle sizes, from just below 2 mm down to fractions of a micrometer, required particular care. Mixing was therefore extended to 4 weeks. The bulk homogeneity of the material was tested by taking subsamples from the drum and analyzing them by X-ray fluorescence spectrometry. As the data analysis offered no indication for material inhomogeneity, the sample was bottled. The bottling procedure was performed as follows: to prevent segregation of fine particles, 10 samples were taken from the centre of the drum immediately upon stopping the rotation and were placed into 10 pre-cleaned brown glass bottles so each contained a minimum of 70 g of soil. The drum was again rotated for a further two minutes and a further 10 samples were subsampled in the same way. The subsampling and bottling operation was continued until 1200 bottles of the soil were obtained. 100 bottles, selected sequentially over the whole bottling process were selected for homogeneity and stability testing.

2.8- New attempts in order to time saving remediation

2.8.1- Ultrasonic Sequential extraction digestion procedure (U-SEP)

One of the main limitations of sequential extraction procedures is that they are extremely time consuming. There has been considerable recent interest in the use of ultrasound to improve, or as an alternative to, mineral acid digestion for the extraction of analytes from environmental samples (Ashley, *et al*., 1999, Davidson, *et al*., 2001). Speeding up sequential extraction by replacing long periods of mechanical shaking with ultrasonic extraction is also attractive. Perez-Cid *et al*. (1998) have successfully developed a modified BCR sequential extraction with the use of focused ultrasound. The extraction of copper, chromium, nickel, lead and zinc from sewage sludge was described, and recoveries similar to those with conventional shaking were obtained. The total extraction time was only 22 min, as opposed to around 50 h with conventional shaking. The same authors have also recently developed an ultrasonic version of the Tessier sequential extraction (Perez-Cid *et al*., 1998; Jamali, *et al*., 2009).

2.8.2- Microwave assisted digestion

The speed and efficiency of instrumentation for reliable determination of trace elements in geological and environmental samples have improved dramatically over the last decades. However, sample preparation methods are still the major factor contributing to the uncertainty in the analytical results (Al- Harahsheh, *et al*., 2009).

Conventional digestion procedures, such as wet digestion and dry ashing, are often the most time consuming stage of the analysis. These methods are labour intensive and tedious, and often have a high contamination potential (Sandorni, *et al*., 2002; Sastre, *et al*., 2002). The improvement of microwaves oven has led to their usage in analytical laboratories for sample digestion. The theory of microwave digestion has been reviewed in detail elsewhere and only brief comment is justified here (Luque-Garcia, *et al*., 2003). The first application of microwaves for sample preparation was reported in 1975 (Abu-Samra, *et al*., 1975). Since that time many microwave assisted dissolution methods have been developed to include a variety of sample matrices such as soil (Melaku, *et al*., 2005), fish (Lopez, *et al*., 2003), sediments (Lo, *et al*., 2005), sludge (Millos, *et al*., 2009) and biological and environmental samples (Sastre, *et al*., 2002) . Microwave digestion procedures are classified according to their operational modes; open vessel microwave assisted digestion, which is more prone to sample contamination, and susceptible to losses of volatile metals and closed (pressurised) vessel procedures, which are rapid and efficient digestion techniques. On line microwave assisted digestion of solid samples and a combination with ultrasonic radiation are also known (Chemat, *et al*., 2004).

A large number of different acid mixtures have been used for microwave digestion. Some methods use HNO_3/HF (Falcina, *et al*., 2000); others use $HClO_4$/HNO_3 (Melaku, *et al*., 2003); HF/HNO_3/HCl (Bettinelli, *et al*., 2000), HNO_3/HCl and HNO_3/H_2SO_4 (Sandroni, *et al*., 2003). In most cases, complete digestion of the sample is required to

achieve reproducible and accurate results (Sandroni, *et al*., 2003). The addition of HF strongly influences the recovery of the microwave acid digestion of environmental samples. This acid breaks down silicates and minerals better than $HClO_4/HNO_3$ and HNO_3/HCl acid combinations. However, HF can give rise to problems in glassware and torch damage of ICP-MS or AAS. This problem can be avoided by using a small volume of HF and addition of saturated boric acid solution, to remove the excess of HF and dilution of samples before analysis.

2.8.2.1- Microwave assisted sequential extraction digestion (MAD-SEP)

The speciation studies of HMs go on to be a large value in environmental monitoring, because not the total amount of the HMs, but more critically their forms presented in the environment that will decide their toxicity, mobility and bioavailability (Bhogal et al., 2003). Several sequential extraction methods have been widely applied to differentiate the chemical forms in which trace metals are present in soils, sediments and sludge. In all sequential extraction schemes, extractants are applied in order to increase reactivity so that the successive fractions obtained correspond to metal association forms with lesser mobility. In sequential BCR extraction (SE), environmental samples (soil, sediment, sewage sludge) are treated with a chain of reagents and determined the elemental concentration into fractions, linked with different mineralogical phases (e.g. carbonates, sulfides and organic matter bound phases) (Quevauviller, *et al*., 2002). The original BCR procedure have been revised due to irreproducibility particularly in reducing extraction ($NH_2OH{\cdot}HCl$) fraction of step 2(Sahuquillo *et al*. (1999) and Rauret *et al*. (1999)). This procedure is very popular during recent years and their application has increased lately, during the certification of reference materials reported (Soylak *et al*., 2004; Mossop, *et al*., 2003; Kazi, *et al*., 2005, Userno, *et al*., 1998). This method is broadly accepted and applied to elemental fractionation in different environmental

samples. But one of the main limits of SE method is that the method is extremely time-consuming. To our knowledge, the use of microwave power for acceleration of SE method with the plan of metal speciation in sewage sludge has not been reported extensively. This microwave power could be introduced to change the magnetic shaking and conventional warming in order to shorten the treatment time.

Microwave power is an extremely useful auxiliary factor, which has lately been exploited for increasing the rate of different chemical processes (Soylak, *et al*., 2006; Filgueiras, *et al*., 2002). A rapid solvent chemical extraction (Ghaedi, *et al*., 2007; Jamali, *et al*., 2009) and metals fractionation in different solid samples (Perez-Cid, *et al*., 2001; Ipolyi, *et al*., 2002) are some of the most known applications. Various microwave oven designs (Perez-Cid, *et al*., 1999) have been used as energy sources and their mechanical parameters have been specifically optimized in order to obtain the best positive operation conditions in each case.

Chapter 3:

Materials

and Method

3. Methodology

3.1- Sample preparation

3.1.1- Shrimp aquaculture sludge

As seafood is one of the major foods in southeast of Asia, there are a lot of shrimp aquaculture farms in this region. At the end of each period of shrimp aquaculture, the resulted sludge is exposed to the air to get dried. The dried sludge is transferred to other places to be used as a soil amendment. It may contaminate the soil by releasing various trace elements into the environment. In this research project, we selected a shrimp aquaculture farm in Selangor, Malaysia and collected four different samples at the site to investigate the mobility of heavy metals by sequential extraction schemes in shrimp aquaculture sludge. In addition, two different sequential extraction procedures (Tessier method and BCR method) were compared for the extraction method of the samples. Furthermore, BCR method was modified for shrimp aquaculture sludge. In our knowledge this is the first time that shrimp aquaculture sludge has been used for sequential extraction in the entire world.

In this work, the effect of changing pH and concentration of hydroxylammonium chloride in step 2 was compared with the unmodified and modified BCR sequential extraction procedure for the determination of potential mobility of heavy metals when applied to shrimp aquaculture sludge samples.

A total of four sludge samples were collected from the bottom of the shrimp aquaculture pond at the end of culture period after discharging wastewater in December 2007 in Selangor, a state in Malaysia. The samples were bagged, labelled and returned to the laboratory where they were coned and quartered to give ~100 g subsamples. Large objects (including stones, pieces of brick, concrete and cinders) were removed and the remaining material was air-dried at the temperature lower than 30 °C under local

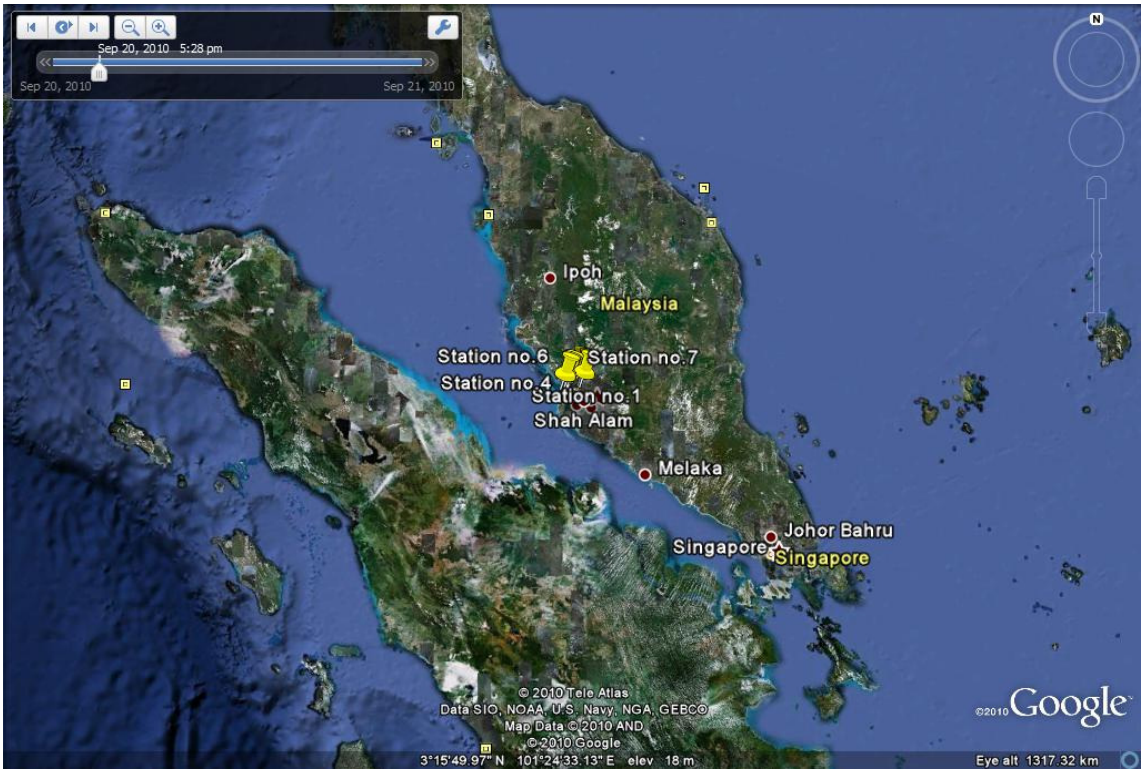
exhaust ventilation in order to remove hazardous volatile components released. After that, the samples were sieved through a 1 mm nylon mesh. The air-dried samples were then coned and quartered again (to ~20 g) and a 1 g of sub-samples was used for sequential extraction.

3.1.2- Sungai Buloh and the straits of Melaka sediment samples

Sungai Buloh is a town in the state of Selangor, Malaysia. It is a 15 minute drive from Sungai Buloh to Kuala Lumpur which is the capital city of Malaysia. The name itself literally means "Bamboo River" in the Malay language.

All the rivers in Kuala Lumpur have their sources in the neighbouring State of Selangor, with the middle reaches going through the city before flowing through Selangor again and out to the sea. In view of that intimate link, freshwater resources are not treated as a separate entity and are still managed by the Selangor Water Works Department. Nevertheless, the highly urban and developed nature of Kuala Lumpur has a major impact on the river systems as they pass through the city. Therefore, it is important to consider the local effects, especially with regard to the utilization and management of the resource.

For investigation of heavy metals mobility potential in different sediments level seven different sampling stations were selected along Sungai Buloh and the straits of Melaka as shown in **Figure 3.1** and **Table 3.1**. Three samples were collected from Sungai Buloh near contaminated sites such as industries and factories and four samples were obtained from the straits downstream of Sungai Buloh. The bottom of some sampling sites was sandy so different amount of some samples were taken at different stations. The samples were divided in 0.5 cm subsamples and used for digestions.



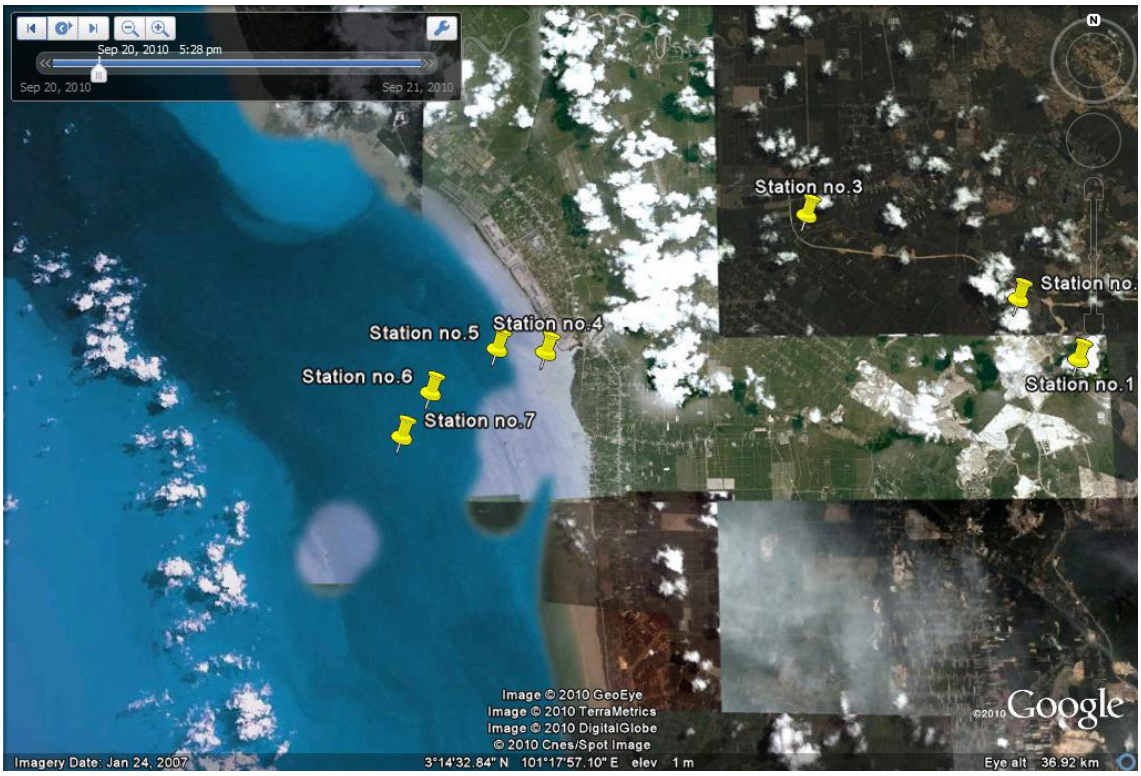


Figure 3.1- Map of the area in which the samples were collected from Sungai Buloh and the straits of Melaka

Table 3.1- Graphical latitude and longitude of the sampling stations

Sampling stations	Latitude	Longitude
1	03°14'47.34"N	101°28'10.38"E
2	03°16'02.64"N	101°27'00.96"E
3	03°17'49.38"N	101°22'42.66"E
4	03°14'56.46"N	101°17'17.76"E
5	03°15'00.60"N	101°16'17.99"E
6	03°14'06.88"N	101°14'57.29"E
7	03°13'13.15"N	101°14'23.19"E

3.1.3- Composting material

For a comparison of microwave assisted digestion and open system digestion, an initial sludge compost was prepared as a mixture of shrimp sludge, plants materials and animal manure at the ratio of 1 (sludge): 4 (crushed bark and husk): 0.5 animal manure (w/w). This mixture was composted in open air and matured in about 6 months as compost 1. Compost 2 consisted of the shrimp sludge co-composted with coco peat waste which has previously used as a growing medium for chilli plant in fertigation system. The air-dried compost samples (20 g) were then coned and quartered and 0.5 g of each sub-sample was employed for both methods of digestion in triplicate.

3.2- Materials

3.2.1- Reagents

3.2.1.1- Reagents used for sequential extraction schemes

All reagents were of analytical reagent grade unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 MΩ/cm resistivity) was used for all dilutions. Acetic acid (glacial, 100%), hydroxyl ammonium chloride, hydrogen peroxide (30 %), ammonium acetate and HNO_3 (69%, sp. gr. 1.42) were of suprapure quality (E. Merck, Fluka). All plastic and glassware were cleaned up by soaking in dilute HNO_3 (1+9) overnight and rinsed with distilled water prior to use. Standard metal solutions (1000 mg L^{-1}) were purchased from Merck (Darmstadt, Germany) or prepared in the lab from pure metals. Extractants were prepared according to the following procedure.

Solution A (acetic acid, 0.11 mol L^{-1}): 25±0.2 mL redistilled glacial acetic acid was added in a fume cupboard to about 500 mL of water in a 1000 mL polyethylene volumetric flask and made up to exactly 1000 mL with further deionised water. 250 mL of this solution (0.43 mol L^{-1} acetic acid) was diluted to 1000 ml to obtain an acetic acid concentration of 0.11 mol L^{-1}.

Solution B (hydroxylamine hydrochloride, 0.1 mol L^{-1}, pH 2.0): 6.95 g of hydroxylamine hydrochloride was dissolved in 900 mL of deionised water. The solution was acidified with concentrated nitric acid to pH 2 and made up to 1000 mL. This solution was prepared on the same day as the extraction was carried out.

Solution C (hydroxylamine hydrochloride, 0.5 mol L^{-1}, pH 1.5): 34.75 g of hydroxylamine hydrochloride, was dissolved in 900 mL of deionised water. The solution was acidified with concentrated nitric acid to pH 1.5 and made up to 1000 mL.

Solution D (hydroxylamine hydrochloride, 0.5 mol L^{-1}, pH 1.0): 34.75 g of hydroxylamine hydrochloride, was dissolved in 900 mL of deionised water. The solution was acidified with concentrated nitric acid to pH 1.0 and made up to 1000 mL.

Solution E (hydroxylamine hydrochloride, 0.7 mol L^{-1}, pH 1.5): 48.65 g of hydroxylamine hydrochloride, was dissolved in 900 mL of deionised water. The solution was acidified with concentrated nitric acid to pH 1.5 and made up to 1000 mL.

Solution F (hydrogen peroxide 8.8 mol L^{-1}): hydrogen peroxide was used as supplied by the manufacturer i.e. acid-stabilized to pH 2.0–3.0.

Solution G (ammonium acetate 1.0 mol L^{-1}): 77.08 g of ammonium acetate was dissolved in 900 mL of deionised water. The solution was acidified to pH 2.0 with concentrated nitric acid and made up to 1000 mL.

3.2.1.2- Reagents for Microwave assisted digestion method

All reagents used such as hydrochloric acid (36% HCl), nitric acid (69% HNO_3) and hydrofluoric acid (48% HF) were of analytical grade (Problab,VWR International, France). These reagents are commonly used for digestion methods of environmental samples. A combination of different volume ratio of mineral acids (HCl and HNO_3 and HF) as a reagent mixture was used to ensure a complete digestion of the samples. HCl and HNO_3 dissolve carbonates (Sastre, et al., 2002), HNO_3 oxidises organic matters (Sastre, et al., 2002), and HF breaks down the aluminosilicate phase (Melaku, et al., 2005; Marin, et al., 2008; McGrath, et al., 1998). The chosen acids were in accordance with previously used methods (Senesil, et al., 1999; Sastre, et al., 2002; Bettinelli, EPA 3052, 1996) and the microwave manufacturer (Multiwave 3000, Anton Paar, Austria) recommendation. Ultrapure water of an 18MΩ/cm resistivity (Milli-Q water purification system, Millipore, USA) was used throughout this work for preparing the solutions and rinsing the vessels. Polypropylene bottles were used for storing the solutions. All glassware and plastic containers used throughout this experiment were immersed in a dilute HNO_3 solution overnight and rinsed with ultrapure water, and finally dried in a clean cabinet. Standards solutions were prepared from a 1000 mol L^{-1} standard for atomic absorption spectroscopy (BDH Chemical, Poole, England) by dilution with acidified ultrapure water (5% (w/w) HNO_3).

3.3- Facilities

3.3.1- Flame Atomic Absorption spectrometry (FAAS)

A flame atomic absorption spectrometer (Perkin Elmer AAnalyst 400 with WinLab 32 Software Version 6.5) was used in this study. All measurements were carried out in an air/acetylene flame. The operating parameters for working elements were set as recommended by the manufacturer. The wavelengths (nm) used for the determination of the analytes were as follows: Cd 228.80; Cr 357.87; Cu 324.75; Zn 213.86; Mn 279.48 and Pb 283.31. The gas flow rate was about 1.10 Lit/min, silt width was between 0.1-2.0 nm and lamp current between 10-20 mA. **Figure 3.2** show an image of Perkin Elmer AAnalyst 400 Flame Atomic Absorption spectrometer. **Table 3.2** shows instrument specific condition of AAS.

Table 3.2- Instrument specific condition of AAS

Element	Wave Length, (nm)	Sens. Check [a] (mg/L)	Linear Range [b] (mg/L)	Min.[c] (mg/L)	Max. [d] (mg/25 mL)	Expected Absorbance Units
Cadmium	228.8	0.5	3.0 - 0.05	0.075	0.0013	0.18-0.36
Calcium	422.7	0.5	3.0 - 0.05	0.0013	0.075	0.25-0.50
Cobalt	240.7	2.5	15.0 - 0.25	0.0063	0.375	0.08-0.16
Copper	324.8	1.5	10.0 - 0.15	0.0038	0.250	0.11-0.22
Iron	248.3	2.5	15.0 - 0.25	0.0063	0.375	0.20-0.40
Manganese	279.5	1.0	5.0 - 0.10	0.0025	0.125	0.22-0.44
Nickel	232.0	4.0	0.40 - 0.01	0.50	20.0	0.18-0.36
Zinc	213.9	0.4	2.0 - 0.04	0.001	0.050	0.20-0.40

[a] Sensitivity Check is the concentration giving approximately 0.2 Absorbance Units (AU).

[b] Linear Range is the upper concentration of linear range.

[c] Minimum mg/L is the concentration giving 0.02 AU (Sens. Check divided by 10)

[d]Maximum is the upper limit in mg per 25 mL (linear range divided by 40).

Fig 3.2- Perkin Elmer AAnalyst 400 Flame Atomic Absorption spectrometer

3.3.2- Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively coupled plasma-optical emission and mass spectrometry (ICP-AES and ICP-MS) are among the most common spectroscopic methods used for determination of heavy metals in environmental samples (Sastre, et al., 2002). ICPMS has emerged as a useful technique for trace analysis of soil owing to its multi elements capability, high detection power and low sample consumption (Falcina, et al., 2000). The drawback of these techniques is that the solid sample required to be transformed into solution prior to the determination of the metal content (Sastre, et al., 2002).

The trace elements determination in Sungai Buloh was performed by an Agilent 7500a ICP-MS (Agilent Technologies, Japan), equipped with a Babington nebulizer, a glass double path spray chamber and a standard quartz torch, and the operating conditions are listed in **Table 3.3.**

Table 3.3- Instrumental parameters for trace elements determination

Parameters	Conditions
ICP-MS	Agilent 7500a
Auto sampler	ASX-500 series
RF power	1350 W
RF matching	1.6 V
Carrier gas flow rate	1.10 L min^{-1}
Peristaltic pump flow rate	0.1 rps

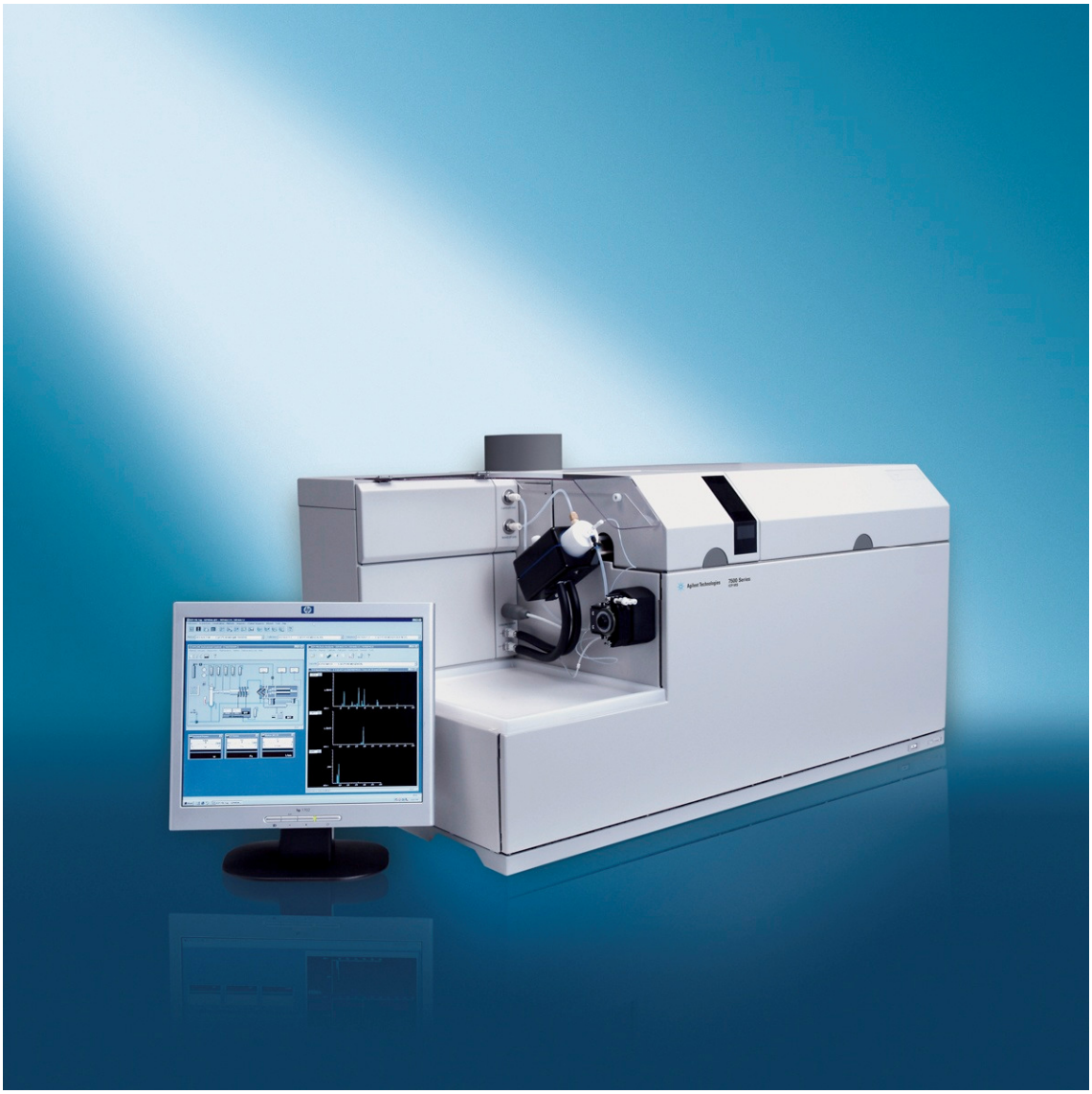


Figure 3.3- Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Agilent Technologies 7500a series

3.3.3- Microwave Assisted System (MAS)

A pressurised closed-vessel Microwave Digestion system (Multiwave 3000, Anton Paar, Austria) as shown in **Figure 3.4** was used to perform microwave assisted digestion procedures for the compost samples. It is equipped with an 8-position rotor (Rotor 8S). The HF vessel consisted of the fluoropolymer liner and the ceramic vessel jacket.

Figure 3.4- Microwave Assisted System Multiwave 3000, Anton Paar, Austria

3.3.4- Hand Core Sediment sampler

For sampling from different level of Sungai Buloh and the straits of Malaka we used a hand core sediment sampler (Wildco, USA) as shown in **Figure 3.5**.

Figure 3.5- Hand core sediment sampler (Wildco, USA)

3.4- Aqua regia digestion

Pseudototal metal content was determined by digestion with aqua regia. One gram of the sample was weighed using a PTFE vessel, and 0.5–1.0 mL of water was added to obtain a slurry. After that 7.0 mL of 12.0 M HCl and then, 2 mL of 15.8 mol L^{-1} HNO_3 was added drop wise to reduce foaming. The PTFE vessels was allowed to stand for 16 h (overnight) at room temperature for slow oxidation of the organic matter in the sample. The solution was heated on a hot plate under the hood. The temperature of the reaction mixture was slowly raised, until reflux conditions were reached and maintained for 2 h. After cooling the PTFE vessel to room temperature, the digests were filtered into a 100-ml volumetric flask and diluted to the mark with deionized water. Each sample was prepared in triplicate.

3.5- Sequential extraction procedures

3.5.1- Tessier sequential extraction method

The Tessier et al. (1979) sequential extraction scheme was employed with the following exceptions: (1) The exchangeable and carbonate phases were extracted in an end-over-end rotator at 8 rpm instead of using a mechanical shaker and, (2) washes after each extraction were not discarded, but added to the supernatant for analyses. All analyses were done in triplicates. 1 g of solid sample was weighed and transferred into a centrifuge tube and the sequential extraction carried out as follows;

3.5.1.1- Exchangeable fraction

1) 8 mL of 1 mol L^{-1} magnesium chloride ($MgCl_2$) at pH 7.0 was added to each centrifuge tube which was then closed tightly and shaken vigorously for mixing. Then, the tubes were placed on an end-over-end rotator at 8 rpm, with continuous agitation at room temperature for 1 hour.

2) The mixtures were taken off and centrifuged for 30 minutes at room temperature.

3) The Supernatants were pipetted and stored in flat-bottom previously labelled centrifuge tubes.

4) Before moving to the next step, the residue was washed with 8 mL of DIW and centrifuged for 30 minutes. The supernatants were pipette and added to the previously labelled centrifuge tubes. This procedure was applied to all the following steps.

3.5.1.2- Carbonate (acid-soluble) fraction

5) 8 mL of 1 mol L^{-1} sodium acetate (NaOAc) that has been adjusted to pH of 5.0 with acetic acid (HOAc) was added to the residue from step one and the centrifuge tubes closed tightly and shaken vigorously for mixing. Then, the tubes were placed on an end-over-end rotator at 8 rpm accompanied by continuous agitation for 5 hours at room temperature.

6) Step 3 and 4 were repeated.

3.5.1.3- Iron and manganese oxides (reducible) fraction

7) 20 mL of 0.04 mol L^{-1} hydroxyl ammonium hydrochloride (NH_2.OH HCl) in 25 % (v/v) acetic acid (HOAc) for 6 hours at 96 ± 3 °C was added to the residues from the second step accompanied by with occasional agitation. This step was done in a water bath.

8) Step 3 and 4 were repeated.

3.5.1.4- Organic matter (oxidizable) fraction

9) 3 mL of 0.02 mol L^{-1} nitric acid (HNO_3) and 5 mL of 30% hydrogen peroxide (H_2O_2) adjusted to pH 2.0 with HNO_3 was added to the residues from the third step.

10) The sample was heated progressively to 85 ± 2°C, accompanied by occasional agitation and the temperature maintained at this level for 2 hours.

11) 3 mL of 30% H_2O_2 adjusted to pH 2.0 with HNO_3 again was added to the mixtures.

12) The solutions were heated at 85°C for 3 hours accompanied by occasional agitation.

13) The solutions were allowed to cool to room temperature.

15) 5 mL of 3.2 mol L^{-1} ammonium acetate (NH_4OAc) in 20% (v/v) HNO_3 was added and diluted to 20 mL with DIW. The centrifuge tubes were closed tightly and shaken vigorously for mixing. Then, the tubes were placed on an end-over-end rotator at 8 rpm, with continuous agitation at room temperature for 30 minutes. The addition of NH_4OAc prevents the extraction of metals onto the oxidized soil.

16) Step 3 and 4 were repeated.

3.5.1.5- Residual fraction

17) The final solid residue was digested with a mixture of hydrofluoric and hydrochloric acid in Teflon beakers. A 0.2 g of sample was first digested with a solution of concentrated HCl (2 mL) and HF (10 mL) and evaporated to near dryness; subsequently a second addition of HCl (1 mL) and HF (10 mL) was made and again the mixture was evaporated to near dryness. Finally, HCl (1 mL) was added and the sample was evaporated until the appearance of white fumes. The residue was dissolved in concentrated nitric acid and diluted to 25 mL as shown in **Table 3.4**.

Table 3.4: Tessier five-stage sequential extraction scheme

Extraction step	Reagent(s)	Nominal target phase(s)
1	$MgCl_2$ 1 mol L^{-1} pH=7	**Exchangable**
2	NaOAc 1 mol L^{-1}, HOAc pH =5	**Carbonatic**
3	$NH_2OH.HCL$ 0.04 mol L^{-1} In 25% HOAc	**Oxides Fe/Mn**
4	H_20_2 8. mol L^{-1}, HNO_3 and NH_4OAc 0.8 mol L^{-1}	**Organic matter and sulphides**
5	$HF/HNO_3/HCl$	Residual

3.5.2- BCR sequential extraction scheme (Sludge sample)

3.5.2.1-Step one

40 ml of acetic acid (0.11 mol L^{-1}) was added to 1 g of the sludge sample in a 100 mL centrifuge tube, stopper and extracted by shaking for 16 h at 22 ± 5°C (overnight). No hold-up time should occur between the addition of the extractant solution and the beginning of the shaking. The extractant was separated from the solid residue by centrifugation at 3000 rpm for 20 min and the resulted supernatant liquid was poured into a polyethylene container and analyzed immediately. The residue was washed by adding 20 mL distilled water, shaking for 15 min on the end-over-end shaker and was centrifuged for 20 min at 3000 rpm. The supernatant was decanted and discarded.

3.5.2.2-Step two

40 mL of a freshly prepared of hydroxylamine hydrochloride (0.1 mol L^{-1}) was added to the residue from step 1 in the centrifuge tube. Resuspend by manual shaking, and then extracted by mechanical shaking for 16 h at 22 ± 5°C (overnight). No delay should occur between the addition of the extractant solution and the beginning of the shaking. The extractant was separated from the solid residue by centrifugation and decantation as in step 1. The extract was retained in a polyethylene container to be analyzed as before. The residue was washed by adding 20 mL distilled water, shaking for 15 min on the end-over-end shaker and centrifuging for 20 min at 3000 rpm. The supernatant was decanted and discarded.

3.5.2.3-Step three

10 mL of hydrogen peroxide (8.8 mol L^{-1}) was carefully added to the residue in centrifuge tube and was digested at room temperature for 1 hour with occasional manual shaking. The digestion was continued for 1 h at 85 ± 2°C, with occasional manual

shaking for the first ½ hour; in a water bath, and then was reduced to the volume less than 3 mL by further heating of the uncovered tube. A further aliquot of 10 mL of hydrogen peroxide was added. The vessel was heated and covered again to 85 ± 2°C and was digested for 1 hour with occasional manual shaking for the first ½ hour. The cover was removed and reduced the volume of liquid to about 1 mL. 50 mL of ammonium acetate (1.0 mol L^{-1}) was added to the cool moist residue and was shaken for 16 h at 22 ± 5°C (overnight). The extract was separated from the solid residue by centrifugation and decantation as in step 1.

3.5.2.4-Step four

The residue was digested with a mixture of acid HF/HNO_3/HCl.

The scheme used in BCR three-stage sequential extraction is summarise in **Table 3.5**.

Table 3.5: BCR three-stage sequential extraction scheme

Extraction step	***Reagent(s)***	***Nominal target phase(s)***
1	HOAc (0.11 mol L^{-1})	**Soil solution, carbonates, Exchangeable metals**
2	$NH_2OH.HCL$ (0. mol L^{-1})	**Oxides Fe/Mn**
3	H_2O_2 (8.8 mol L^{-1}) then NH_4OAc (1.0 mol L^{-1}) at pH=2	**Organic matter and sulfides**
Residual*	**HF/HNO_3/HCl**	**Remaining, non-silicate bound metals**

* Digestion of the residual material is not a specification of the BCR protocol.

3.5.3- Modification of BCR sequential extraction procedure (Sludge sample)

For the modification of BCR sequential extraction procedure the extractions were performed using the reagents given in **Table 3.6**.

3.5.3.1- Step one

40 mL of acetic acid 0.11 mol L^{-1} (Solution A) was added to a 1 g of sludge sample in a 100-mL centrifuge tube stopper and extracted by shaking for 16 h at room temperature (overnight). No hold-up should occur between the addition of the extractant solution and the beginning of the shaking. The extractant was separated from the solid residue by centrifugation at 3000 rpm for 20 min and the resultant supernatant liquid was transferred into a polyethylene volumetric flask and analyzed immediately. The residue was rinsed by adding 20 mL of distilled water, shaking for 15 min on the end-over-end shaker and was centrifuged for 20 min at 3000 rpm. The supernatant was decanted and discarded.

3.5.3.2-Step two

40 mL of a freshly prepared of hydroxyl ammonium chloride (refer to Solutions B, C, D or E) was added to the residue from step 1 in the centrifuge tube, resuspended by manual shaking, and then extracted by mechanical shaking for 16 h at room temperature (overnight). No delay should occur between the addition of the extractant solution and the beginning of the shaking. The extractant was separated from the solid residue by centrifugation and decantation as in step 1. The extract was kept in a polyethylene volumetric flask to be analyzed as before. The residue was washed by adding 20 mL distilled water, shaking for 15 min on the end-over-end shaker and centrifuging for 20 min at 3000 rpm. The supernatant was decanted and discarded.

3.5.3.3-Step three

10 mL of 8.8 M hydrogen peroxide (Solution F) was carefully added to the residue in the centrifuge tube and digested at room temperature for 1 h with occasional manual shaking. The digestion was continued for 1 h at 85±2 °C, with occasional manual shaking for the first 1/2 h; in a water bath, and then reduced to a volume less than 3 ml by further heating of the uncovered tube. A further aliquot of 10 ml of hydrogen peroxide was added. The tube, with cover, was heated again at 85±2 °C and digested for 1 h with occasional manual shaking for the first 1/2 h. After that, the cover was removed and the volume of liquid was reduced to about 1 mL. Then 50 ml of 1.0 M ammonium acetate (solution G) was added to the cool moist residue and shaken for 16 h at room temperature (overnight). The extract was separated from the solid residue by centrifugation and decantation as in step 1.

3.5.3.4-Step four

The residue remaining at the end of the step 3 was digested as the same aqua regia and HF digestion.

Table 3.6: Comparison between unmodified and modified BCR method

Step	Fraction	Nominal target Phase(s)	Procedure A (original BCR)	Procedure B	Procedure C	Procedure D (modified BCR)
1	Exchangeable, water and acid soluble	Soluble species, carbonates, cation exchange sites	0.11 mol L^{-1} acetic acid	0.11 mol L^{-1} acetic acid	0.11 mol L^{-1} acetic acid	0.11 mol L^{-1} acetic acid
2	Reducible	Iron and manganese oxyhydroxides	0.1 mol L^{-1} hydroxyl ammonium chloride at pH 2	0.5 mol L^{-1} hydroxyl ammonium chloride at pH 1	0.7 mol L^{-1} hydroxyl ammonium chloride at pH 1.5	0.5 mol L^{-1} hydroxyl ammonium chloride at pH 1.5
3	Oxidizable	Organic matter and sulphides	Hydrogen peroxide followed by 1.0 mol L^{-1} ammonium acetate at pH 2	Hydrogen peroxide followed by 1.0 mol L^{-1} ammonium acetate at pH 2	Hydrogen peroxide followed by 1.0 mol L^{-1} ammonium acetate at pH 2	Hydrogen peroxide followed by 1.0 mol L^{-1} ammonium acetate at pH 2
4	Residual		Aqua regia + HF	Aqua regia + HF	Aqua regia + HF	Aqua regia+HF

3.6-Microwave assisted digestion procedures (MAD)

In this study we report the comparison of open system digestion and microwave assisted digestion in two different composts of shrimp aquaculture sludge which used as organic growing media for crop. Our first step was to validate the methods using certified reference materials (CRMs) of soil origin and to propose a rapid, safe, and best sample preparation method. The second step was to apply the method for monitoring heavy metals content in the composts. To the best our knowledge, this is the first research in analysis of shrimp aquaculture sludge by microwave assisted digestion using different solvent volume in the entire world. Microwave PTFE vessels were cleaned using 10 mL of concentrated HNO_3, heating for 15 min at 180 ºC (800W) and then rinsed with ultrapure water followed by heated for 15 min at 180 ºC before each digestion. PTFE evaporation vials were soaked overnight in dilute HNO_3 and then rinsed with ultrapure water. All samples were accurately weighed to approximately 0.5 g directly in microwave vessels. Relative volumes of each mineral acid were derived from the aqua regia digestion procedure with small changes as summarized in **Table 3.7**. The same amount of HNO_3 (6 mL) with different volumes of HF and HCl were used for the digestion in procedures A and B. In procedure A, the same amount of HCl and HF (1 mL each) was added to HNO_3, giving a total of 8 mL of an acid mixture.

The volume of both acids (HCl and HF) was increased twice with the amount of HNO_3 (6 mL) as procedure B. In procedures C and D, 6 mL of HCl was mixed with different volumes of HNO_3 and HF. However, the same ratio of HNO_3 and HF was employed in these procedures. The only difference between procedures C and D was that procedure C used less amount of HNO_3 and HF compared to procedure D. The digestion program itself consisted of a 10-min gradual increase in temperature to 200 ºC, a 15-min step at 200 ºC (1000W; 106 Pa) and then a ventilated cooling stage. This program was chosen

in agreement with manufacturer recommendations and earlier studies on microwave assisted digestion optimisation (Sandroni, et al., 2002; Melaku, et al., 2005; Zhou, et al., 1995). After cooling to room temperature, all the digests from the each procedure (**Table 3.7**) were filtered through a 0.45µm PTFE filter and then evaporated on a hot plate at 60 ºC. Evaporation was a necessary step since acid concentrations would have been too high for the AAS (atomic absorption spectrometry) and would have required dilutions to take place to such an extent that trace element could not be detected. Care was taken to avoid burning of the evaporation residues. All solutions were diluted to 50 mL with ultrapure water and stored in polyethylene vials at 4 ºC until analysis using AAS. Blanks were treated in the same way without sample for all procedures. This procedure completed in 75 min.

Table 3.7: Reagents, volumes and operating condition for microwave digestion (MAD) methods

Step	Procedure	Reagents (ml)	Power (W)	Hold time (min)	Pressure (Pa)	Temperature (˚C)
1	A	HNO_3 (6 mL), HCl (1 mL), HF(1 mL)	1000	15	10^6	200
2	B	HNO_3 (6 mL), HCl (2 mL), HF(2 mL)	1000	15	10^6	200
3	C	HNO_3 (1 mL), HCl (6 mL), HF(1 mL)	1000	15	10^6	200
4	D	HNO_3 (2 mL), HCl (6 mL), HF(2 mL)	1000	15	10^6	200
Vent				50		

3.7-Open (conventional) wet acid digestion procedure (OD) (Compost sample)

A mixture of mineral acids as tabulated in **Table 3.7** was employed in an open system digestion method. The determination of trace elements content followed the procedure recommended by the International Organization for Standardization (ISO 11466, 1995). 0.5 g of each triplicate compost sample with a total volume of 8 ml or 10 ml of an acid mixture (HNO_3 +HF + HCl) were added into a digestion flask covered with a watch glass and heated in a sand bath heater for 16 h. After cooling at room temperature, the digests was filtered through a 0.45µm PTFE filter paper into a 50-mL volumetric flask and diluted with ultrapure water containing 5% HNO_3 to the mark. Finally, all solutions were kept in a refrigerator at 4 ℃ until analysis using AAS.

3.8- Speciation of heavy metals in sediment by modified BCR, ultrasound and microwave assisted sequential extraction procedure for CRM BCR 701

We investigated that if microwave heating techniques were applicable to the dissolution of total metals from different solid matrixes within very low time period as compared to conventional methods, the microwave techniques could also be used to stimulate the rapid release of heavy metals in each of the different chemical binding fractions of soils, solid waste and sediments. So the main objective of this work was to apply and compare time-saving extraction device (microwave oven) in the BCR three-step sequential extraction procedure, with the principal aim of reducing the 51 h treatment time, whilst at the same time maintaining, from an environmental point of view, the high recovery values as provided by the conventional BCR sequential procedure. Speeding up of the SE method has been previously approved out with the use of ultrasound power (Kazi, *et al*., 2006). The use of simple and widely available extraction device, such as a domestic microwave oven, was investigated. The microwave oven procedure was optimized using a soil amended with sewage sludge reference material, with target values in each step being those obtained by the application of the conventional BCR procedure using mechanical shaker. For the quality control of the analytical performance and the validation of the newly developed method, the conventional and optimized alternative extraction methods were compared using the analysis of BCR 701. Microwave operation parameters (heating time and power) were optimized for Cu, Cr, Ni, Pb and Zn and in each case the most optimum conditions were selected. Flame atomic absorption spectrometry (FAAS) were used for the measurement of metals in the extracts. Modified sequential extraction method (MSE) is broadly accepted and applied to elemental fractionation in different environmental samples. The main limitations of sequential extraction procedures are that, they are extremely time-consuming, and are less used for routine analysis. This problem has also been noted and is replacing the

conventional procedure by other alternatives, such as microwave (MW) heating and ultrasonic (US) shaking. Davidson and Delevoye have effectively developed a modified BCR sequential extraction with the use of ultrasound energy (Davidson, *et al.*, 2001) It is well known that exposure of solids to ultrasound leads to structural changes in their surface morphology (Ipoly, *et al.*, 2002). The impact of the ultrasound jet and its associated shock wave erodes the solid, leading to localized fragmentation of particles (Greenway, *et al*., 2002); the achieved results were comparable to what can be achieved with mechanical shaking, while the time required (20–30 min per step) was much shorter (Kazi, *et al*., 2006). The sequential extraction procedure could also be improved by using MW irradiation, recently applied for acceleration of different chemical processes (Mahan, *et al*., 1987), including multi-step sequential extraction methods (Perez-Cid*, et al*., 1999). So this energy could be introduced to replace the conventional and magnetic shaking in order to shorten the treatment time. Microwave power is an extremely useful auxiliary factor, which has lately been exploited for increasing the rate of different chemical processes (Soylak, *et al*., 2006; Filgueiras, *et al*., 2002). A rapid solvent chemical extraction (Ghaedi, *et al*., 2007; Mahan, *et al*., 1987) and metals fractionation in different solid samples (Perez-Cid, *et al*., 2001; Ipoly, *et al*., 2002) are some of the most known applications. Various microwave oven designs (Perez-Cid, *et al*., 1999) have been used as energy sources and their mechanical parameters have been specifically optimized in order to obtain the best positive operation conditions in each case. We investigated that if microwave heating techniques were applicable to the dissolution of total metals from different solid matrices within very low time period as compared to conventional methods, the microwave techniques could also be used to stimulate the rapid release of heavy metals in each of the different chemical binding fractions of soils, solid waste and sediments. The extracting solution has been listed in **Table 3.8**.

Table 3.8: The extracting solutions of Modified BCR, Ultrasonic and Microwave assisted digestion methods

	Fraction 1	**Fraction 2**	**Fraction3**
M-BCR	1.0 g sample + 40 mL of 0.11 mol L^{-1} CH_3COOH Shaking =16 h	40 mL 0.5 mol L^{-1} NH_2OH-HCl pH=1.5 Shaking time=16 h	10 mL H_2O_2 (8.8 M) heat to 85°C for 1 h and add 5 ml H_2O_2 heat to 85°C for 1 h and then add 50 mL CH_3COONH_4 (1 mol L^{-1} pH=2)
USE	Replicate six 1.0 g sample + 40 mL of 0.11 mol L^{-1} CH_3COOH Sonication=0-60 min	40 mL 0.5 mol L^{-1} NH_2OH-HCl pH=1.5 Sonication=0-60 min	10 mL H_2O_2 (8.8 M) heat to 85°C for 1 h and add 5 mL H_2O_2 heat to 85°C for 1 h and then add 50 mL CH_3COONH_4 (1 mol L^{-1}, pH=2) Sonication=0-60 min
MSE	Replicate six 0.25 g sample + 10 mL of 0.11 mol L^{-1} CH_3COOH Microwave induced time 20-120 s	10 mL 0.5 mol L^{-1} NH_2OH-HCl pH=1.5 Microwave induced time 20-120 s	2.5 mL H_2O_2 (8.8 M) heat to 85°C for 1 h and add 1.25 mL H_2O_2 heat to 85°C for 1 h and then add 12.5 mL CH_3COONH_4 (1 mol L^{-1}, pH=2) Microwave induced time 30-120 s

Chapter 4: Results and discussion

Chapter 4: Results and discussion

In this project we investigate two different samples. Sample 1 is a sample from shrimp aquaculture sludge and sample 2 is a sum of some samples from Sungai Buloh Sediments.

4.1- Shrimp aquaculture sludge

4.1.1- Pseudototal metal digestion

The results of Pseudototal metal digestion are presented in **Table 4.1** and the amounts of heavy metals in 4 samples are compared in **Figure 4.1**. The major heavy metal components in the aquaculture shrimp sludge were Ca, Fe, Mn and Zn. In comparison with standards of heavy metals in soils, the concentrations of all of the heavy metals in this study except Cd were less than the standards. Although the concentration of Cd is less than other heavy metals in the sludge it is very harmful if the sludge to be used as a soil amendment. The results of direct digestion of heavy metals show that with the control of Cd the sludge can be used as an organic medium in soils for agriculture but it is better for fruitless plants such as rubber tree.

Table 4.1: Concentration of heavy metals by pseudo-total metal digestion (µg g^{-1})

no. of sample	Zn	Fe	Cu	Mn	Pb	Cr	Cd	Ca
1	158.65	2958.08	14.55	741.16	37.41	56.67	8.18	4127.54
2	157.11	3028.9	14.88	693.94	39.72	54.29	8.05	4255.94
3	155.44	3068.62	15.05	705.33	37.12	56.07	8.16	4071.72
4	160.32	2919.36	14.72	729.77	39.43	53.69	8.29	4200.12

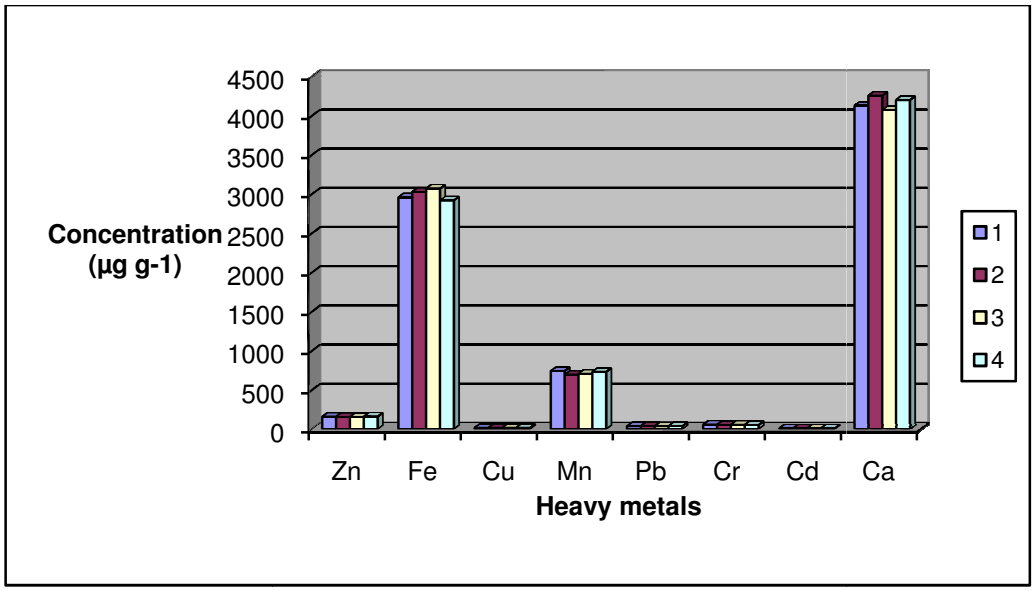


Figure 4.1: Concentration of heavy metals in sludge by Pseudototal digestion

4.1.2- Sequential Extraction Schemes

In shrimp aquaculture sludge two different sequential extraction methods (Tessier and BCR) have been compared. Also BCR sequential extraction method has been modified.

The accuracy of the method was checked by recovery studies. The recovery values for five stages have a variety between 94.42 and 107.59% and for three stages BCR methods between 96.14 and 105.26%. The results of this study provide important information on the potential of the trace metals. The results predicted that all heavy metals are mainly associated with residual forms.

4.1.2.1- Tessier five-step and BCR three-step methods

The average of concentration of heavy metals in four samples by Tessier method and BCR method were presented in **Table 4.2 and Table 4.3**, respectively. All metal concentrations (except Cd, Zn and Cu) were extracted to be higher in residual fraction in this method. The proportion of the metals in the exchangeable and carbonate bound in five stages method followed the order: Mn>Fe>Zn>Pb>Cr>Cd>Cu (**Table 4.2 and Table 4.3**).

According to these methods also the percent of trace metals found in exchangeable and reducible fraction were very low (except Cd and Mn). Among the metals studies Mn was extracted predominantly in the first and second steps of Tessier method and the first step of BCR method. It represents the metal bound to the exchangeable and reducible phases. On the other hand, only very small fractions of other elements were present as fractions 1 and 2 in five stages method and fraction 1 in BCR method. These results are similar to other studies by Tuzen et al. (2003). Manganese is bound to the non residual and residual fraction. This element is mainly present in the acid-soluble fraction (carbonate form) and shows significant affinity for the residual fraction. Soluble

Table 4.2: Concentration of heavy metals by Tessier five stages method (µg g^{-1}), N=3

Heavy metals	Zn	Fe	Cu	Mn	Pb	Cr	Cd
First step	3.48 ± 0.25*	22.36 ± 1.97	0.35 ± 0.01	121.52 ± 4.25	3.18 ± 0.10	0.13 ±0.01	2.76 ± 0.03
Second step	5.85 ± 1.24	31.83 ± 1.82	0.56 ± 0.04	188.51 ± 0.06	4.37 ± 0.51	5.89 ± 0.27	0.95 ± 0.01
Third step	36.64 ± 0.09	311.48 ± 2.63	0.48 ± 0.01	71.85 ± 14.09	6.53 ± 0.19	2.32 ± 0.01	2.97 ± 0.10
Fourth step	59.47 ± 0.39	452.67± 3.31	8.19 ± 0.01	33.85 ± 5.08	2.95 ± 0.19	10.45 ± 0.01	1.16 ± 0.03
Residual	49.32 ± 1.21	2137.45 ± 1.86	5.77 ± 1.18	279.78 ± 0.66	19.44 ± 0.76	33.31 ± 0.31	0.95 ± 0.04
Four step+ Residual	154.76 ± 3.18	2955.79 ± 11.59	15.35 ± 1.25	695.51 ± 24.14	36.47 ± 1.75	52.10 ± 1.49	8.79 ± 0.18
Pseudo-total	157.88 ± 2.44	2993.49 ± 74.11	14.80 ± 0.25	717.55 ± 23.61	38.42 ± 1.30	55.18 ± 1.49	8.17 ± 0.12
Recovery (%)	98.02	98.74	103.71	96.92	94.92	94.42	107.59
C_f	2.13	0.38	1.66	1.48	0.88	0.56	8.25

*** Mean ± SD**

Table 4.3: Concentration of heavy metals by BCR method (µg g^{-1}), N=3

Heavy metals	Zn	Fe	Cu	Mn	Pb	Cr	Cd
First step	7.73 ± 0.52*	45.51 ± 4.26	0.92 ± 0.38	321.25 ± 9.32	5.57 ± 0.32	8.17 ± 0.24	4.07 ± 0.03
Second step	39.95 ± 3.47	329.44 ± 23.04	0.97 ± 0.14	74.69 ± 8.46	7.99 ± 0.34	5.02 ± 0.09	2.66 ± 0.04
Third step	58.09 ± 0.22	465.46 ± 9.09	7.27 ± 0.13	38.93 ± 1.86	3.60 ± 0.02	11.11 ± 0.11	1.00 ± 0.01
Residual	53.13 ± 2.28	2152.04 ± 3.41	5.07 ± 0.36	295.09 ± 9.24	21.38 ± 3.37	30.38 ± 0.71	0.87 ± 0.01
Three step+ Residual	158.90 ± 6.49	2992.45 ± 39.80	14.23 ± 1.01	729.96 ± 28.88	38.54 ± 4.05	54.58 ± 1.15	8.60 ± 0.09
Pseudo-total	157.88 ± 2.44	2993.49 ± 74.11	14.80 ± 0.25	717.55 ± 23.61	38.42 ± 1.30	55.18 ± 1.49	8.17 ± 0.12
Recovery (%)	100.64	99.96	96.14	101.72	100.31	98.91	105.26
C_f	1.99	0.39	1.80	1.47	0.80	0.80	8.88

* **Mean ± SD**

manganese released during this process may then be co-precipitated with the $CaCO_3$ carbonates (Kersten, 1988). The manganese reduction zone is earlier established than this of the iron reduction zone and suggests less reducing condition for this element.

Iron is essentially bound to the residual fraction, suggesting relatively low mobility of this element in both methods. This was confirmed previously by Caplat *et al*. (2005) that a sharp decrease of the dissolved iron concentration from the surface to the deeper layer. This drop can be attributed to oxidizing condition, which control the solubility of iron since the dissolved form of Fe(II) is very unstable and easily precipitable.

Copper shows a marked presence in forms bound to organic matter and sulfides. The solubility and mobility of this element is therefore controlled by organic matter mineralization. Elderfield (1981) suggested that in main sediments, 80% of copper would be linked to the colloidal organic matter, and Skrabal et al. showed that this element would be complexed to organic ligands (Caplat *et al*. 2005; Elderfield, 1981; Skrabal, *et al*., 2000). Copper was found mostly in bound to organic matter or sulfides fractions (about 52%). Copper is a chalcophilic element, it is mainly bound to sulfides in nature. It has been shown in other studies that an increase in the solubility of copper is due to oxidation (Bruder-Hubscher, *et al*., 2002; Weisz, *et al*., 2000; Tuzen *et al*. 2003). Takalioglu *et al*. (2000) employed four steps (BCR) sequential procedure to lake sediment samples. They reported that the highest concentration values (about 50%) for copper was found at oxidizable fraction. We found the same result in the present work. This might be due to the high tendency of Cu to be absorbed on organic matter (Alloway, 1995).

Lead mostly was extracted in residual condition (about 53%) in both methods. The concentration of lead in the exchangeable/ carbonates bounds was just about 15%. Also concentration of this element in Fe/Mn oxides fraction was only 18% in these methods. This value is in agreement with that reported in other studies (Tessier, *et al*., 1979).

Zinc is suggested as the most labile metal because of its stronger affinity to non residual fraction, already observed in other works (Perez-Cid, *et al*., 1996). The amount of exchangeable and organic zinc in sediment found is very low in other studies reported by Tessier et al. (1979). The fractional composition of sludge zinc shows the highest extraction of zinc in oxidation reaction in these methods (about 38%).

Cd was extracted predominantly in the exchangeable and reducible fractions. About 80% of Cd was extracted in these two fractions. These results have been shown in other studies (Arian, et al., 2008). Cr was found mostly in residual fraction (about 63%). Also it was extracted mainly in organic and sulphides bound (about 20%).

The precision of the proposed BCR extraction methods (expressed as RSD %) was found in the range of (3.99–9.6%) for all metals.

4.1.2.2- Mobility potential of heavy metals

The mobility and immobility and thus toxicity of heavy metals in waste depend largely on their type of binding forms. **Tables 4.2 and 4.3** compare the mobility potential of heavy metals in different forms. It was noticed that Cd and Mn have the highest ability and susceptibility to be released from the sludge by the simple ion exchanged mechanism. It is seen that the chloride content in the leachate can bind with Cd, Mn and enhances its mobility in the solid waste (Kjeldsen, *et al*., 2002).

The presence of acid soluble portion of Mn and Pb indicates its sensitivity to the acidic condition and tendency to leach easily.

However, in the case of Fe, it was found relatively insensitive to acid condition. This result is in agreement with other study for high mobility of Mn in the acid environment (Watmough *et al*., 2007).

Under varied reduction conditions, the release of Cd, Zn and Cu from the solid waste sludge is expected. This is in contrast with Cu and Zn which precipitates with sulphide, absorbed on the organic matter, and appears to be stable under the anaerobic condition.

4.1.2.2.1- Contamination factor (C_f)

The determination of contamination factor of heavy metals is an important aspect that indicates degree of risk of heavy metals to environment in relation with its retention time. A high contamination factor of heavy metal shows low retention time and high risk to the environment. The individual contamination factor (C_f) of heavy metals was used to estimate the relative retention time of heavy metals retained in the sludge. It is determined by dividing the sum of concentration of each heavy metal in the mobile phase (non-residue phase) by its concentration in the residue phase. The global contamination factor (C_f) is equal to the sum of individual factor (Prechthai, *et al*., 2008; Femandes, 1997; Borona, *et al*., 1999).

Tables 4.4 and 4.5 show estimated contamination factor of each metal in the sludge. The calculated factors in both methods show highest ability of Cd, Zn, Cu and Mn to be released from the sludge of aquaculture shrimp, whereas Fe and Cr have the lowest. The residual concentration of any heavy metal is considered non-mobile fraction and is an important part influencing the mobility nature of the heavy metal. The combined effect of Cd, Zn, and Cu in high concentration and with high mobility potential shows the increased possible risk of these metals to the environment.

The high proportion of metals in the residual fraction and the generally low levels of extractable metals indicate that the sediment is relatively unpolluted. Metals bound to reducible and organic fractions are presumed to be more bioavailable and thus more harmful to biota. Trace elements in unpolluted soils or sediments are mainly bound to silicates. In environmental studies the determination of the different ways of binding gives more information on trace metal mobility, as well as on their availability or toxicity, in comparison with the total element content (Rauret, 1998). **Figures 4.2 and 4.3** shows the percent of heavy metals in different fractions for both methods.

Table 4.4: Mobility of heavy metals in sludge with Tessier method

step	condition	mobility
1	Ion exchange	**Cd> Mn> Pb> Cu> Zn>Fe> Cr**
2	Acid dissolution	**Mn> Pb> Cr> Cd> Zn>Cu> Fe**
3	Reduction reaction	**Cd> Zn> Pb> Fe> Mn> Cr> Cu**
4	Oxidation Reaction	**Cu> Zn> Cr> Fe >Cd > Pb >Mn**
5	Insolubility	**Fe> Cr> Pb> Mn> Cu> Zn> Cd**

Table 4.5: Mobility of heavy metals in sludge in BCR method

step	condition	mobility
1	Ion exchange	**Cd> Mn> Pb> Cu> Zn>Fe> Cr**
2	Acid dissolution	**Mn> Pb> Cr> Cd> Zn>Cu> Fe**
3	Reduction reaction	**Cd> Zn> Pb> Fe> Mn> Cr> Cu**
4	Oxidation Reaction	**Cu> Zn> Cr> Fe >Cd > Pb >Mn**
5	Insolubility	**Fe> Cr> Pb> Mn> Cu> Zn> Cd**

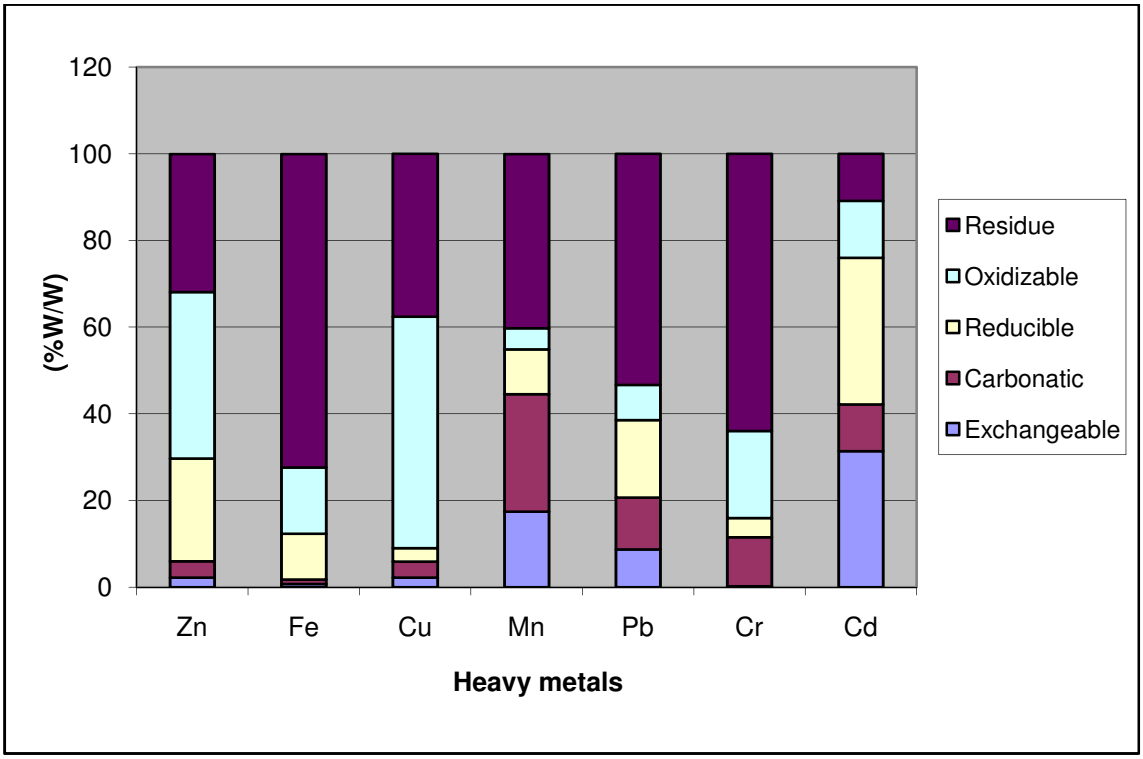


Figure 4.2: The percentage of heavy metal by Tessier method

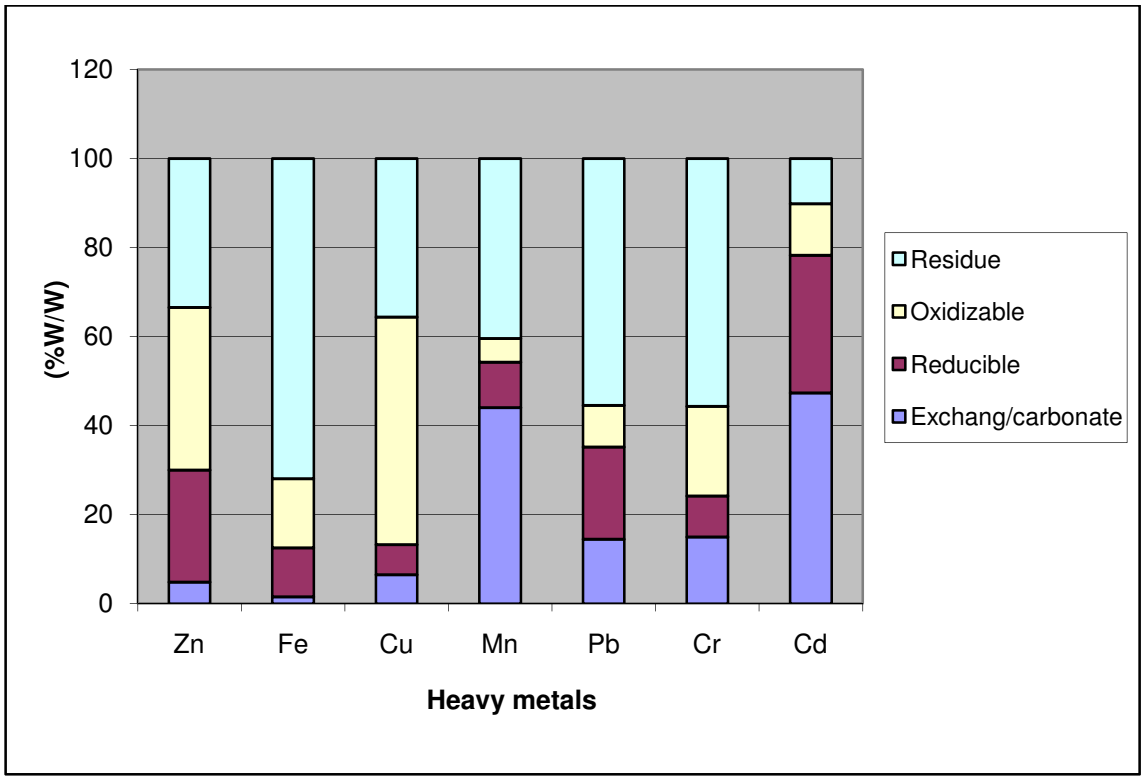


Figure 4.3: The percentage of heavy metal by BCR method.

4.1.2.2.2- Risk assessment code (RAC)

The risk assessment code, defined as the fraction of metal exchangeable and/or associated with carbonates (% F1 for BCR and % F1+%F2 for Tessier), was determined for the seven trace metals, and the values interpreted in accordance with the RAC classifications. This classification is described by Perin et al. (1985).

Metals are bound to different sediment fractions, with the strength of the binding determining their bioavailability and the risk associated with their presence in aquatic systems. The risk assessment code (RAC) was determined based on the percentage of the total metal content that was present in the first sediment fraction in BCR method (% F1) and fraction 1+ Fraction 2 in Tessier method, where binding is weak and the metals pose a greater risk to the aquatic environment (Jain, 2004).When this percentage is less than 1%, the sediment is of no risk to the aquatic environment. Percentages of 1–10% reflect low risk, 11–30% medium risk, and 31–50% high risk. Above 50%, the sediment poses a very high risk, and is considered dangerous, with metals easily able to enter the food chain (Jain, 2004; Perin et al. 1985) .

Figure 4.2 shows the results of RAC with values given as percentage of the fraction soluble in acid and carbonate fraction (% F1 + % F2).

In Tessier method we can see a low risk for Zn, Fe and Cu. Pb and Cr show a medium risk but Mn and Cd have a high risk in this method.

Figure 4.3 illustrates the results of the risk factor analysis, with values given as percentages of the fraction soluble in acid (% F1) for the seven trace metals in BCR.

In this method also we can see same trend of results. In general, the sediments show a medium risk for most metals with RAC values greater than 11%, but a high risk indicate for Cd and Mn.

4.1.3- A modification of the BCR sequential extraction procedure to investigate the potential mobility of copper and zinc in shrimp aquaculture sludge

In this work, the effect of changing pH and concentration of hydroxyl ammonium chloride in step 2 was compared with the unmodified and modified BCR sequential extraction procedures for the determination of potential mobility of heavy metals when applied to shrimp aquaculture sludge samples.

Extractions were performed using the reagents given in **Table 3.5**.

Triplicate measurements of four samples obtained from shrimp aquaculture sludge were subjected to four different conditions in step 2 of BCR sequential extraction schemes. The results showed a good agreement between triplicate measurements of each sample. An internal check was performed on the results of the sequential extraction by comparing the total amount of metals removed in the procedure with the results of the pseudototal digestion (**Table 4.6**).

The recovery of the sequential extraction procedure was obtained as follows:

Recovery= {(Step 1+Step2+Step3+Residual)/ Pseudototal} ×100

The average recovery values for four different sequential extraction procedures were in the range 80–101% and 85–97% for Zn and Cu respectively.

Table 4.6: Mean concentration of Cu and Zn in fractionation by different conditions of BCR sequential extraction procedure (µg g^{-1}). N=3

Sample	Method	Step 1	Step2	Step3	Step 4	Sum	Pseudo-total	Recovery (%)	C_f
Zn	A	7.73±0.52*	39.95±3.47	58.09±0.22	53.13±2.28	158.90±6.49	157.88±2.44	100.64	1.99
	B	7.62±0.66	24.02±1.78	53.12±0.49	49.59±0.31	134.35±3.24	157.88±2.44	85.09	1.71
	C	7.14±0.51	16.65±0.72	56.62±1.65	46.33±1.11	126.74±3.99	157.88±2.44	80.27	1.73
	D	7.52±0.51	50.42±3.37	51.14±1.39	47.68±1.95	156.76±7.22	157.88±2.44	99.29	2.28
Cu	A	0.92±0.38	0.97±0.14	7.27±0.13	5.07±0.36	14.23±1.01	14.80±0.25	96.14	1.80
	B	0.84±0.13	0.89±0.27	6.81±0.79	5.63±0.55	14.17±1.74	14.80±0.25	95.74	1.52
	C	0.40±0.07	0.65±0.05	7.28±0.84	4.23±0.72	12.56±1.68	14.80±0.25	84.86	1.97
	D	0.49±0.11	0.94±0.24	6.78±1.32	6.21±0.76	14.42±2.43	14.80±0.25	97.43	1.31

* **Mean ± SD**

4.1.3.1- Exchangeable and acid soluble fraction

The fraction shows an increase in the amount of heavy metal that would be released into the environment when conditions became more acidic. It is this fraction which gives adverse impact to the environment (Usero *et al*., 1998; Long *et al*., 2009). In this work, the level of zinc is between 7.14± 0.51µg g^{-1} and 7.73±0.52 µg g^{-1}. About 4–6% of the total zinc was released in 4 methods. Approximately between 3–6.5% of Cu was released in acid soluble fraction.

4.1.3.2- Reducible fraction

Reducible fraction of heavy metal represents the contents of metal bound to iron and manganese oxides that would be released if the substrate is subjected to more reductive conditions (Panda, et al., 1995). In the present study, we focused on changing the pH and the concentration of hydroxylammonium chloride and consideration of percentage recovery. In method A, we applied the unmodified BCR sequential extraction on shrimp aquaculture sludge. In this method 39.95±3.47 μg g^{-1} zinc and 0.92±0.38 μg g^{-1} copper were released in reducible fraction (**Table 4.6**). The percentage recovery in method A is about 100.64 and 96.14 for Zn and Cu respectively. In step 2 of unmodified BCR sequential extraction scheme, 25.14% of zinc and 6.81% of copper were released (**Figures 4.4 and 4.5**).

In method B, the concentration of hydroxyl ammonium chloride had been increased to 0.5 M and the pH of solution lowered to 1.0.

The level of zinc in this method decreased significantly to 24.02±1.78 μg g^{-1}. However, the amount of copper only decreased slightly to 0.89±0.27 μg g^{-1}. Correspondingly, the extractable percentage decreased to 17.86% for Zn and 6.28% for Cu (**Figures 4.4 and 4.5**).

In method C, the concentration of hydroxyl ammonium chloride had been increased to 0.7 M and pH of solution increased to 1.5. The recovery and concentration of both elements highly in this fraction decreased again. The percentages of recoveries were decreased to 80.27% and 84.86% for Zn and Cu respectively. (**Table 4.6**)

In method D, the concentration of hydroxyl ammonium chloride was decreased to 0.5 mol L^{-1} and pH of solution was kept at 1.5. In this method, the concentration of zinc was increased significantly but in contrast, copper partitioning was relatively unaffected by this method when compared with the unmodified BCR sequential extraction.

Method D recovered more zinc in step 2 than method A. Nevertheless, method A obtained higher recovery compare to methods B and C, indicating that although the reductant concentration was increased to 0.5 mol L^{-1} from 0.1 mol L^{-1} would increase the percentage of recovery with a decrease in the concentration of hydroxyl ammonium chloride. Furthermore, the reduction of pH to 1.5 gave the best recovery for Zn but for Cu, the unmodified BCR sequential extraction scheme showed a high percentage recovery.

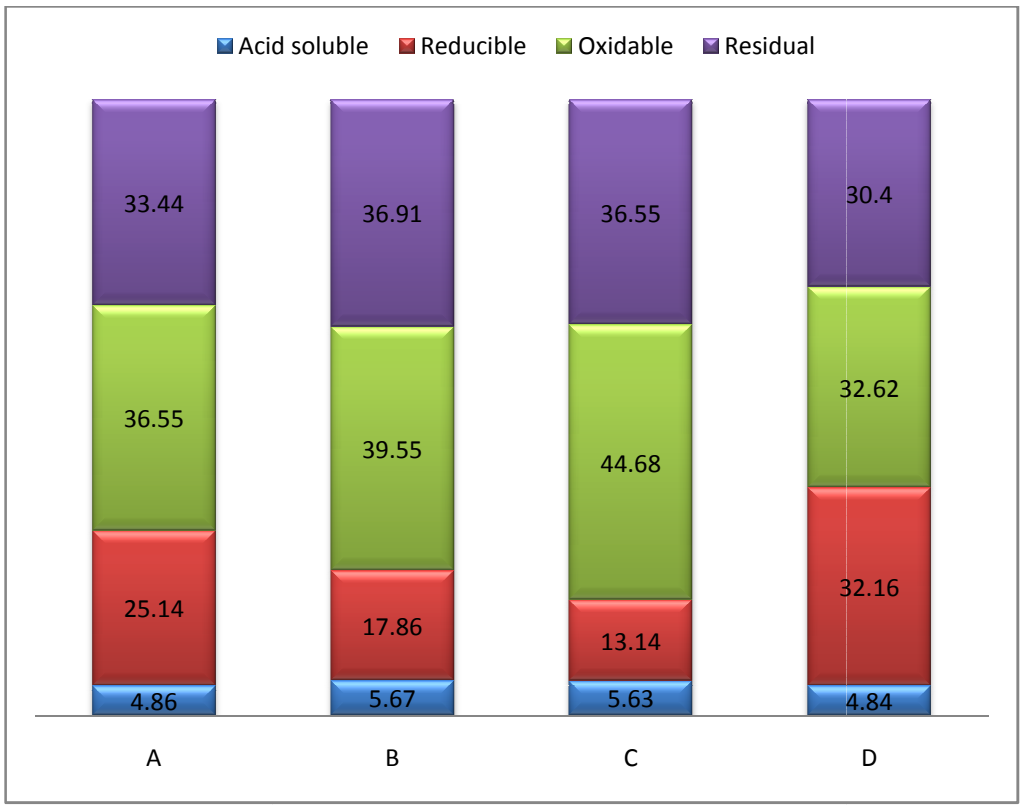


Figure 4.4: The percentage of Zn in each fraction by different methods.

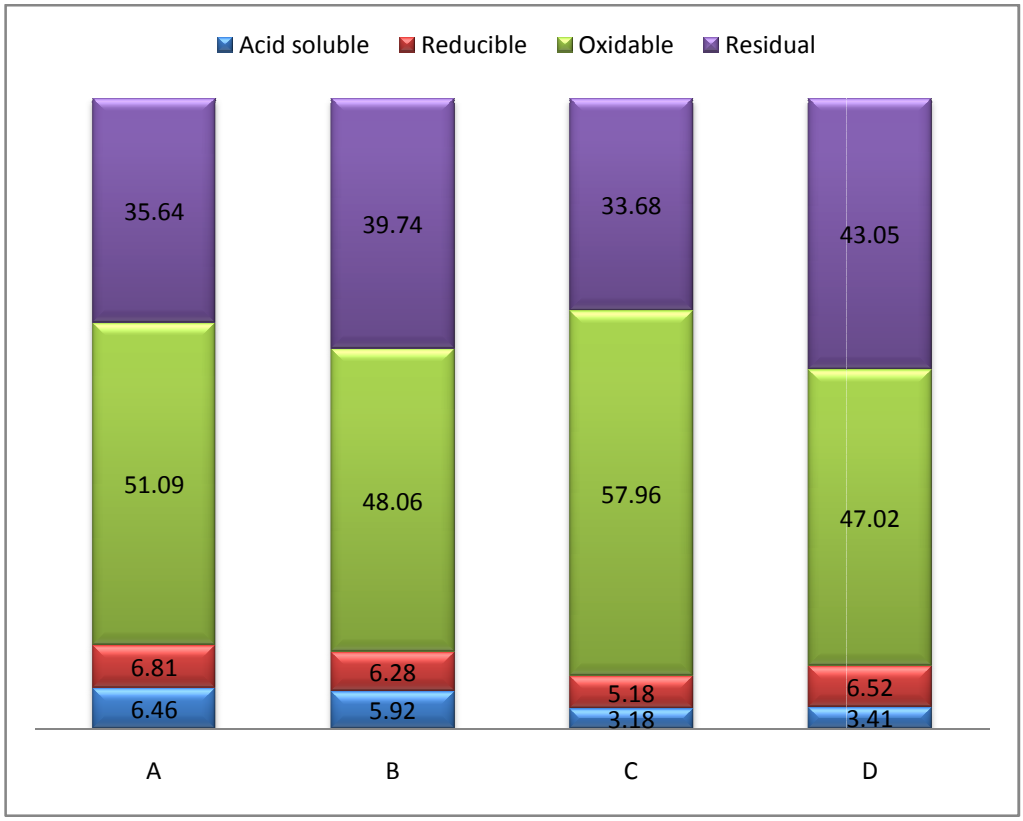


Figure 4.5: The percentage of Cu in each fraction by different methods.

4.1.3.3-Oxidizable fraction

Oxidizable fraction shows the amount of heavy metal bound to the organic matter and sulphur which would be released into the environment if condition became oxidative. As shown in **Table 4.6** and **Figures 4.4 and 4.5**, both Cu and Zn were mostly extracted under oxidizing condition (32.62%–44.68% for Zn and 47.02%–57.96% for Cu). It has been shown in other studies that an increase in the solubility of copper and zinc is due to oxidation.

4.1.3.4- Residual fraction

The residual fraction represents the metals that have strongest association with the crystalline structures of the minerals. It is always not easy to extract the metals in this fraction. In this work, for all four methods, the residual fractions were extracted using aqua regia. The residual fraction of shrimp aquaculture sludge was contained 30.40%–36.91% of Zn and 33.68%–43.05% of Cu. The residual fraction of both elements in shrimp aquaculture sludge was calculated for second extractable percentage in all fractions. It was found that Zn and Cu were stable in this sample because both elements were bound to organic matter and sulphurs and the crystalline structures of the minerals.

4.1.4- Comparison of unmodified and modified BCR sequential extraction schemes for the fractionation of heavy metals in shrimp aquaculture sludge

4.1.4.1- BCR sequential extraction procedure (Methods A and B)

The samples collected from shrimp aquaculture sludge were subjected to two different conditions in step 2 of the BCR sequential extraction schemes. The results showed good agreement between triplicate measurements of each sample. An internal check was performed on the results of the sequential extraction by comparing the total amount of metals removed in the procedure with the results of the pseudo-total digestion (**Table 4.7 and 4.8**). The recovery of the sequential extraction procedure was accounted as follows:

$$\text{Recovery} = \{(\text{Step 1}+\text{Step2}+\text{Step3}+\text{Residual})/\ \text{Pseudo-total}\} \times 100$$

4.1.4.1.1- Acid extractable/exchangeable fraction

This fraction reflects the amount of heavy metals that would be released into the environment when conditions become more acidic. It is this fraction that gives adverse impact to the environment (Long et al. 2009). In this study, about 50% of total of Cd and Mn, and 15% of total Cr and Pb were released in this fraction in both Methods A and B. **Figures 4.6 and 4.7** show the percentages of heavy metals released in this fraction. This study also revealed that this fraction constituted the smallest fraction with respect to the distribution of all metals except for Cd and Mn.

4.1.4.1.2- Easily reducible fraction

This fraction represents the concentration of metal bound to iron and manganese oxides that would be released if the substrate was subjected to more reductive condition (Panda et al. 1995). As shown in **Figures 4.6 and 4.7**, Cd has the highest portion in reducible fraction among the seven metals determined.

The proportional distribution is in the order of Cd > Zn > Pb > Fe ≅ Mn > Cr > Cu.

The results of this study revealed that the major portion of Cd in sludge samples was extracted in exchangeable and reducible fractions, namely, 50% and 31%, respectively. Therefore, Cd in this aquaculture sludge is more labile and hence easily released into the environment and available for uptake by plants and by aquatic organisms.

The amount of Cd extracted in the reducible fraction increased significantly from 31% to 41% when modified BCR sequential extraction method was used as compared to that obtained using the unmodified BCR sequential extraction method, while the amount of Zn increased from 25% to 30%. Moreover, the amount of Pb increased slightly from 21% to 24%. In general, all metals showed an increase in the percentages extracted when using the modified method except for Cu which decreased slightly from 6.8% to 6.6%. Therefore, an increase in the concentration of hydroxyl ammonium chloride in step 2 has a significant effect on the release of certain heavy metals. Furthermore, a reduction of pH might have also decrease the readsorption of metals from the liquid phase.

4.1.4.1.3- Oxidizable fraction

The oxidizable fraction represents the amount of metal bound to the organic matter and sulphides that will be released into the environment if conditions become oxidative. As shown in **Table 4.7 and 4.8**, and **Figures 4.7 and 4.8** and, Cu, Zn, and Cr existed predominantly in the oxidizable fraction (Cu, 49%; Zn, 37%; Cr, 20%) of the sludge samples, implying that Cu, Zn, and Cr are associated with organic matter or present as sulphides.

4.1.4.1.4- Residual fraction

The metals in the residual fraction are strongly bound to the crystalline structures of the minerals. These metals are not easily extracted or removed. In both Methods A and B, the residual fraction was attacked with aqua regia. Our results revealed that Fe and Mn have the highest concentrations in this fraction as compared with other heavy metals (**Table 4.7 and 4.8**). Furthermore, Fe, Pb, and Cr have the highest concentrations in residual fraction as compared to other fractions. Therefore, the potential mobility of these metals from shrimp aquaculture sludge is quite low.

Table 4.7: Heavy metals distributions and contamination factors by unmodified BCR method, N=3

Heavy metals ($\mu g\ g^{-1}$)	**Zn**	**Fe**	**Cu**	**Mn**	**Pb**	**Cr**	**Cd**
Step 1	7.73 ± 0.52*	45.51 ± 4.26	0.92 ± 0.38	321.25 ± 9.32	5.57 ± 0.32	8.17 ± 0.24	4.07 ± 0.03
Step 2	39.95 ± 3.47	329.44 ± 23.04	0.97 ± 0.14	74.69 ± 8.46	7.99 ± 0.34	5.02 ± 0.09	2.66 ± 0.04
Step 3	58.09 ± 0.22	465.46 ± 9.09	7.27 ± 0.13	38.93 ± 1.86	3.60 ± 0.02	11.11 ± 0.11	1.00 ± 0.01
Step 4(Residual)	53.13 ± 2.28	2152.04 ± 3.41	5.07 ± 0.36	295.09 ± 9.24	21.38 ± 3.37	30.38 ± 0.71	0.87 ± 0.01
Three step+ Residual	158.90 ± 6.49	2992.45 ± 39.80	14.23 ± 1.01	729.96 ± 28.88	38.54 ± 4.05	54.58 ± 1.15	8.60 ± 0.09
Pseudo-total	157.88 ± 2.44	2993.49 ± 74.11	14.80 ± 0.25	717.55 ± 23.61	38.42 ± 1.30	55.18 ± 1.49	8.17 ± 0.12
Recovery (%)	100.64	99.96	96.14	101.72	100.31	98.91	105.26
C_f	**1.99**	**0.39**	**1.80**	**1.47**	**0.80**	**0.80**	**8.88**

* **Mean ± SD**

Table 4.8: Heavy metals distributions and contamination factors by modified BCR method, N=3

Heavy metals ($\mu g\ g^{-1}$)	**Zn**	**Fe**	**Cu**	**Mn**	**Pb**	**Cr**	**Cd**
Step 1	7.73 ± 0.52*	45.51 ± 4.26	0.92 ± 0.38	321.25 ± 9.32	5.57 ± 0.32	8.17 ± 0.24	4.07 ± 0.03
Step 2	50.42 ± 3.37	351.35 ± 20.11	0.94 ± 0.14	92.27 ± 6.19	9.51 ± 0.62	8.41 ± 0.59	4.06 ± 0.28
Step 3	58.09 ± 0.22	465.46 ± 9.09	7.27 ± 0.13	38.93 ± 1.86	3.60 ± 0.02	11.11 ± 0.11	1.00 ± 0.01
Step 4(Residual)	53.13 ± 2.28	2152.04 ± 3.41	5.07 ± 0.36	295.09 ± 9.24	21.38 ± 3.37	30.38 ± 0.71	0.87 ± 0.01
Three step+ Residual	169.37 ± 6.39	3014.36 ± 36.87	14.20 ± 1.01	747.54 ± 26.61	40.06 ± 4.33	58.07 ± 1.65	10.00 ± 0.33
Pseudo-total	157.88 ± 2.44	2993.49 ± 74.11	14.80 ± 0.25	717.55 ± 23.61	38.42 ± 1.30	55.18 ± 1.49	8.17 ± 0.12
Recovery (%)	107.27.64	100.70	95.94	104.18	104.26	105.23	122.40
C_f	**2.19**	**0.40**	**1.80**	**1.53**	**0.87**	**0.91**	**10.49**

* **Mean ± SD**

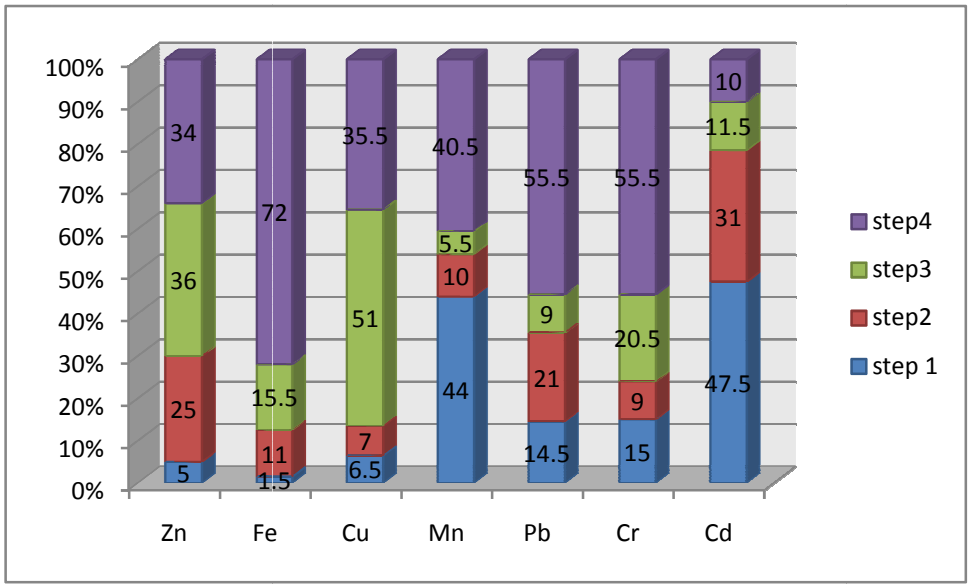


Figure 4.6: The percentages of heavy metals released in the four fractions by unmodified BCR

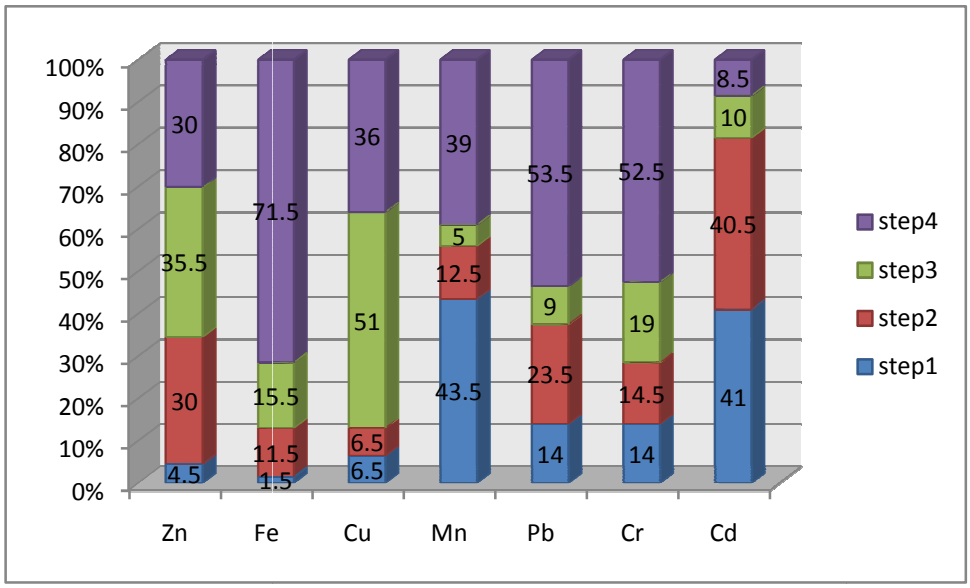


Figure 4.7: The percentages of heavy metals released in the four fractions by modified BCR

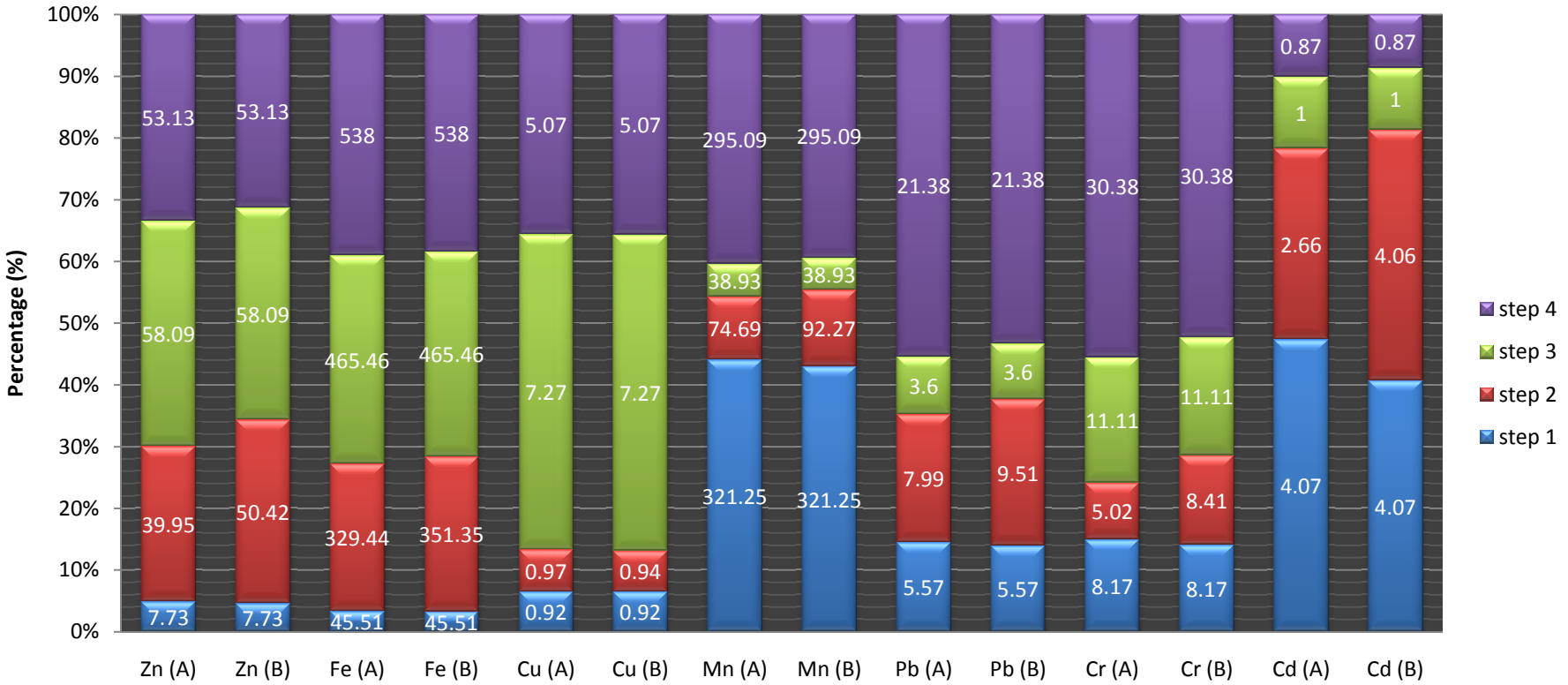


Figure 4.8: Comparison between percentages of heavy metals released using Method A and B

4.1.4.2- Effect of modification of BCR sequential extraction procedure

Modification of step 2 of the BCR sequential extraction procedure was made by increasing the concentration of hydroxyl ammonium chloride from 0.1 to 0.5 mol L^{-1} and subsequently reducing the pH of the reagent from 2.0 to 1.5. As shown in **Figure 4.8**, there is a significant effect of releasing heavy metals in this reducible fraction for most of the metals except Cu. Method B released more of these elements as compared with Method A.

Our results also indicate that similar amount of all elements were released in other fractionation steps of sequential extraction for both methods.

According to our previous studies (Nemati et al. 2009), it was found that an increase in the concentration of hydroxyl ammonium chloride itself without reducing the pH of the extractant might not significantly enhance the recovery of heavy metals. This may indicate that the ability to prevent readsorption is more important than an increase in the amount of reductant present.

4.1.4.3- Comparison with indicative values

Reference sample was extracted to assess the quality of the data obtained. Lake sediment reference material BCR CRM 701 was used. The results obtained were in close agreement with indicative values and are generally acceptable (**Table 4.9**).

Table 4.9: Comparison between indicative and found (*F*) values for extractable trace element in lake sediment reference material (BCR CRM 701).

Steps	Cd	Cd (F)	Cr	Cr (F)	Cu	Cu (F)	Pb	Pb (F)	Zn	Zn (F)
Step 1	7.34	7.11	2.26	2.16	49.3	48.5	3.18	3.01	205	207
Step 2	3.77	3.59	45.7	43.9	124	117	126	122	114	109
Step 3	0.27	0.25	143	139	55.2	53.5	9.3	8.9	45.7	42.9

4.1.4.4- Environmental implications

The individual contamination factors (C_f) of elements in shrimp aquaculture sludge were calculated in order to study heavy metals retention. In this study, C_f was calculated as the sum of the concentrations of heavy metals extracted in the first three steps of the sequential extraction methods divided by the concentration in the residual fraction (Jamali et al. 2007). The experimental results indicated that among the unmodified and modified sequential extraction methods, Cd, Zn, Cu, and Mn gave the highest C_f values as shown in **Tables 4.7 and 4.8**.

4.1.5- Comparative study on open system digestion and microwave assisted digestion methods for metal determination in shrimp sludge compost

4.1.5.1- Method validation

Microwave assisted digestion (MAD) (**Table 3.6**) and open system digestion (OD) techniques were compared to determine the capability of the methods to be used in sample preparation of compost samples herein, taking into account the information obtained when comparing the recovery, the total time taken for analysis, the operational difficulties, the amount of acid used and the safety requirements during the process.

Tables 4.10 and 4.11 show the results obtained for Zn, Cu, Cr, Ni, Pb and Cd in the analysis of the certified reference material BCR 146-R. Triplicate measurements were performed using both MAD and OD methods. During the AAS analysis, a procedure blank and spike samples involving all reagents were run to check cross contamination and interferences for each set of every ten samples.

The accuracy assessment revealed that the recoveries of MAD method ranging from 88.7% to 95.5%, 95.7% to 98.8%, 82.1% to 95.6% and 87.7% to 97.2% for procedures A–D, respectively.

The recovery of each metal was calculated based on the mean value for CRMs [(measured concentration ($\mu g\ g^{-1}$)/mean CRM certified value ($\mu g\ g^{-1}$))×100]. Our results indicate that the lowest recoveries of MAD were found in procedure C for Cr (82%). The best recoveries were found in B for all metals.

For OD method, the best recoveries were found in procedure B. Nevertheless, when compared between MAD and OD methods the highest recoveries were found in MAD except for Zn. The obtained result in procedure B for both digestion systems was in good agreement with the certified values. Comparison between procedures A and B in MAD for determination of metals in the analysis of the certified reference material

(BCR 146-R) showed that the addition of 1ml HCl and 1ml HF, the recoveries of all metals were increased intensively.

Our results revealed that an aqua regia with the ratio of 3:1 (HNO_3/HCl) is the best combinations of acid used in microwave digestion. More concentrated HNO_3 was required for the digestion as the sludge samples contained more organic materials. The sludge samples should be treated with HF, assuming that some heavy metals might have deposited on silicate compounds. Furthermore, the metals might be released into an aquatic phase in the environment.

The ratio of 1: 3(HNO_3/HCl) (in method D showed that) the recoveries of all metals were significantly decreased except for Pb. These results showed that trace elements in the CRM 146 sludge samples could be released easily with HNO_3 as the matrix consisted of organic materials.

The results of the open digestion system are found to be similar to that of microwave assisted digestion. Nevertheless, the recoveries obtained from microwave assisted digestion are better than that of open system digestion. Perhaps, this is due to lost and oxidation of some elements in open system digestion. More HF required in these samples since silicate base as a matrix of these samples.

With comparison between these two digestion systems it could be deduced that in most cases microwave assisted digestion extraction was sufficient to quantify the level of concentrations of these trace elements in the soil and also seemed to be a more attractive procedure from the point of low acid consumption, shorter digestion time, and safety coupled to a good accuracy. Consequently, this investigation was done to evaluate the best acid combinations for digestion of compost shrimp samples for use in further experiments.

Table 4.10: Concentration of metals in CRM 146-R using microwave assisted system digestion (MAD) (µg g^{-1}). N=3

	Method	Zn	Cu	Cr	Ni	Pb	Cd
CRM (Certified)		3060± 60*	838± 16	196± 7	70± 5	609± 14	**18.8± 0.5**
CRM (Found)	A	2923± 39	796± 21	174± 9	63± 8	571± 14	**17.9± 0.7**
Recovery (%)		**95.5**	**95.0**	**88.7**	**90.0**	**93.7**	95.2
CRM (Found)	B	3025± 64	824±17	190±16	67±8	596± 13	**18.3±.8**
Recovery (%)		**98.8**	**98.3**	**96.9**	**95.7**	**97.9**	97.3
CRM (Found)	C	2888± 49	777± 11	161± 12	61± 9	582± 17	**16.9± 1.2**
Recovery (%)		**94.3**	**92.7**	**82.1**	**87.2**	**95.6**	89.9
CRM (Found)	D	2902± 59	782± 18	172± 15	63± 9	592± 22	**17.2± 1.4**
Recovery (%)		**94.8**	**93.3**	**87.7**	**90.0**	**97.2**	91.5

* **Mean ± SD**

Table 4.11: Concentration of metals in CRM 146-R using open system digestion (OD) (µg g^{-1}), N=3

	Method	Zn	Cu	Cr	Ni	Pb	Cd
CRM (Certified)		3060± 60*	838± 16	196± 7	70± 5	609± 14	18.8± 0.5
CRM (Found)	A	2906± 48	777± 28	168± 9	59± 8	573± 22	17.0± 1.3
Recovery (%)		**94.6**	**92.7**	**85.7**	**84.2**	**94.1**	**90.4**
CRM (Found)	B	3031± 52	818± 21	188± 13	66± 7	590± 12	18.1± 0.8
Recovery (%)		**99.0**	**97.6**	**95.9**	**95.1**	**96.9**	**96.2**
CRM (Found)	C	2791± 55	713± 26	165± 22	56± 9	561± 24	16.6± 1.6
Recovery (%)		**91.2**	**85.1**	**84.2**	**80.0**	**92.1**	**88.3**
CRM (Found)	D	2803± 43	702 ± 16	159 ± 19	58 ± 8	549 ± 20	16.9 ± 2.1
Recovery (%)		**91.6**	**83.7**	**81.1**	**82.8**	**90.1**	**89.9**

* **Mean ± SD**

4.1.5.2- Analysis of real samples

The development method for both microwave assisted digestion and open system digestion were applied for analysis of two different composts (Sample 1 and 2) as shrimp aquaculture sludge compost by atomic absorption spectrometry. The mean concentration of each element studied has shown in **Tables 4.12 and 4.13**.

4.1.5.2.1- Microwave assisted digestion method (MAD)

The elemental concentration of six metals measured in shrimp aquaculture sludge compost using microwave assisted digestion with different volumes of reagents were found in the range of 271–373 µg–1 for sample 1 and 267–352 µg g^{-1} for sample 2 (Zn), 13–17 µg g^{-1} for sample 1 and 13–20 µg g^{-1} for sample 2 (Cu), 60–89 µg g^{-1} for sample 1 and 52–82 µg g^{-1} for sample 2 (Cr), 20–37 µg g^{-1} for sample 1 and 16–32 µg g^{-1} for sample 2 (Ni), 166–216 µg g^{-1} for sample 1 and 155–202 µg g^{-1} for sample 2 (Pb), 7.1–9.3 µg g^{-1} for sample 1 and 6.7–8.8 µg g^{-1} for sample 2 (Cd), respectively (refer **Table 4.12**).

4.1.5.2.2- Open system digestion

In this digestion system, the concentrations of trace elements by using different solvents were analyzed (refer **Table 4.12**). Although the recoveries obtained for all trace elements in method B for open system digestion were similar to microwave assisted digestion, the percentage recoveries obtained from the microwave digestion were better. The concentration of trace elements were in the range of 281–335 $\mu g\ g^{-1}$ for sample 1 and 263–302 $\mu g\ g^{-1}$ for sample 2 (Zn), 13–19 $\mu g\ g^{-1}$ for sample 1 and 11–16 $\mu g\ g^{-1}$ for sample 2 (Cu), 56–78 $\mu g\ g^{-1}$ for sample 1 and 51–67 $\mu g\ g^{-1}$ for sample 2 (Cr), 21–33 $\mu g\ g^{-1}$ for sample 1 and 17–31 $\mu g\ g^{-1}$ for sample 2 (Ni), 153–197 $\mu g\ g^{-1}$ for sample 1 and 155–182 $\mu g\ g^{-1}$ for sample 2 (Pb), 6.8–8.2 $\mu g\ g^{-1}$ for sample 1 and 6.0–7.9 $\mu g\ g^{-1}$ for sample 2 (Cd), respectively.

The concentration of Cd, Pb and Zn are found higher than the world soil mean values (Reimann *et al*., 2000) but in comparison with allowable concentrations of trace metals considered in agriculture soil by the European Community (Lucho-Constantino et al., 2005), except Cd. A remediation should be taken to remove such metal for sustainable practices in agriculture.

The results obtained in this work revealed that the concentration of trace elements in sample 1 is mostly higher than sample 2 in both method digestions, suggesting the sources of the metals were mostly obtained from the sludge itself and not from other materials (crushed bark, husk, coco peat and animal manure) used for the composting.

The source of water used for the shrimp aquaculture should be monitored and treated at the early stage of breeding process (from irrigated or storage pond) in order to decrease the level of heavy metals in sludge during the harvest period (Nemati *et al*., 2009). The texture of the soil used (sandy of peat) for the aquaculture activities also will contribute to the high level of the heavy metals in the shrimp sludge (Nemati *et al*., 2009).

Our previous studies showed that shrimp sludge contained significantly high level of zinc, calcium, nitrate and phosphate, indicating that the remediation sludge waste has a potential to be used as an organic medium for growing crop especially for rubber plantation or other fruitless crop (Nemati *et al*., 2009). At the same time, it will solve the problem related to disposal of shrimp sludge from aquaculture pond.

Effects of different solvent volume and digestion systems on elements concentration for both samples have been shown in **Figures 4.9-4.12**.

Comparison between two digestion methods with different volumes of acid mixture (HCl–HNO_3–HF) revealed that method B included of 6 ml HNO_3, 2 mL of HCl and 2 mL of HF was the best acid mixture used in both microwave and open system digestions methods for the compost samples analyzed by AAS in this work. Moreover, open system digestion took 16 h for complete reflex digestion whereas, microwave assisted system required less than 75 min for the complete digestion.

Table 4.12: Mean concentration of metals with different solvent using microwave assisted system digestion (µg g^{-1}). N=3

Samples	Method	Zn	Cu	Cr	Ni	Pb	Cd
Sample 1	A	343 ± 26*	17 ± 1.0	86 ± 15	32 ± 8	207 ± 15	7.2 ± 1.3
	B	373 ± 14	21 ± 2.6	89 ± 9	37 ± 12	216 ± 21	9.3 ± 1.1
	C	277 ± 12	13 ± 1.1	62 ± 15	20 ± 5	171 ± 26	7.1 ± 0.5
	D	271 ± 22	14 ± 1.3	60 ± 15	23 ± 8	166 ± 18	7.3 ± 0.7
Sample 2	A	330 ± 19	16 ± 1.2	80 ± 14	28 ± 6	191 ± 21	6.8 ± 0.6
	B	352± 23	20 ± 2.5	82 ± 14	32 ± 9	202 ± 19	8.8 ± 0.2
	C	270 ± 17	13 ± 2.2	52 ± 10	18 ± 6	159 ± 16	6.7 ± 0.5
	D	267 ± 14	13 ± 1.5	54 ± 11	16 ± 5	155 ± 25	6.9 ± 0.3

* **Mean ± SD**

Table 4.13: Mean concentration of metals with different solvent ratio used in open system digestion method (µg g^{-1}).N=3

	Method	Zn	Cu	Cr	Ni	Pb	Cd
Sample 1	A	333 ± 17*	17 ± 1	69 ± 11	30 ± 6	178 ± 26	7.4 ± 0.6
	B	335 ± 18	19 ± 2	78 ± 13	33 ± 9	197 ± 22	8.2 ± 1.1
	C	281 ± 17	13 ± 2	56 ± 13	21 ± 4	159 ± 28	6.8 ± 0.3
	D	285 ± 22	13 ± 1	58 ± 17	24 ± 7	153 ± 20	7.1 ± 0.5
Sample 2	A	301 ± 21	16 ± 2	63 ± 10	31 ± 5	162 ± 20	7.5 ± 0.8
	B	322 ± 16	16 ± 2	67 ± 7	29 ± 6	182 ± 14	7.9 ± 0.9
	C	266 ± 18	11 ± 1	51 ± 8	18 ± 4	155 ± 17	6.1 ± 0.6
	D	263 ±22	11 ± 1	53 ± 10	17 ± 6	159 ± 15	6.0 ± 0.9

* **Mean ± SD**

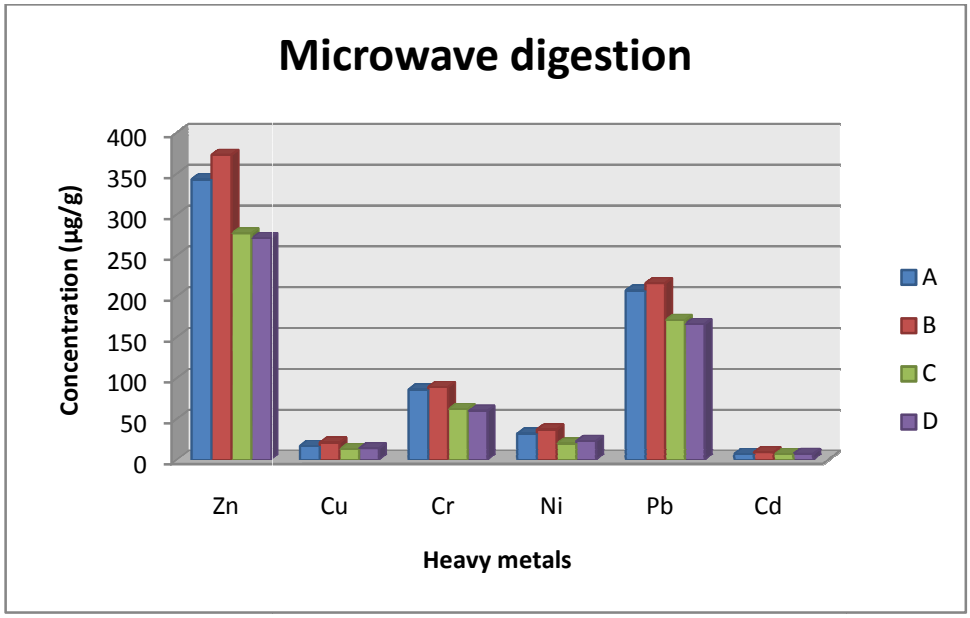


Figure 4.9: Effects of different solvent volume and systems digestion on elements concentration in sample 1 by microwave digestion
(A) 6 mL HNO_3 + 1 mL HCl + 1 mL HF
(B) 6 mL HNO_3 + 2 mL HCl + 2 mL HF
(C) 1 mL HNO_3 + 6 mL HCl + 1 mL HF
(D) 2 mL HNO_3 + 6 mL HCl + 2 mL HF
Sample 1: the ratio of 1 (shrimp sludge): 4 (crushed bark and husk): 0.5 animal manure (w/w).

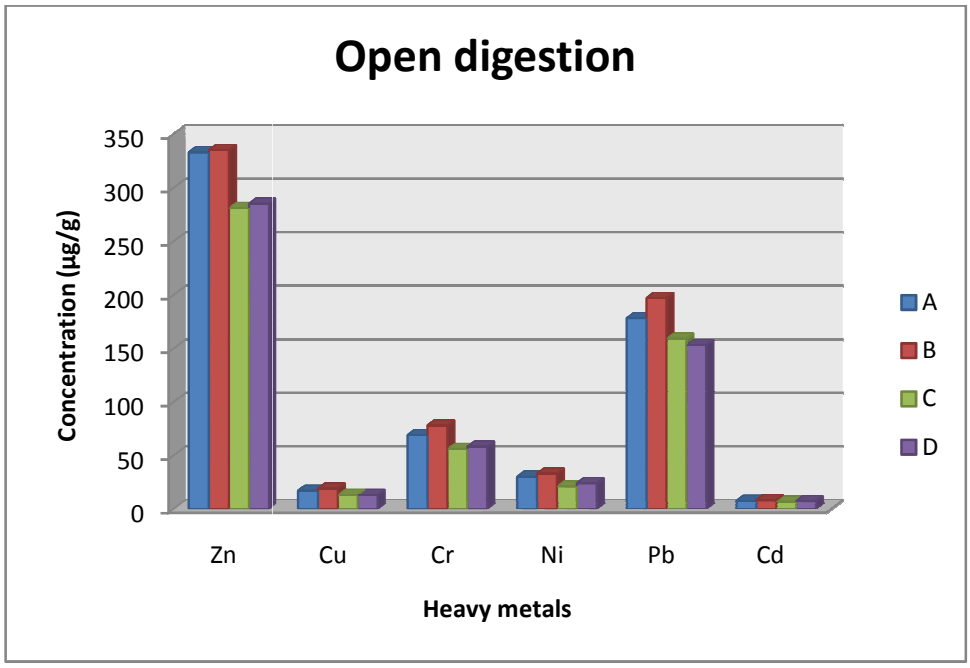


Figure 4.10: Effects of different solvent volume and systems digestion on elements concentration in sample 1 by open digestion
(A) 6 mL HNO_3 + 1 mL HCl + 1 mL HF
(B) 6 mL HNO_3 + 2 mL HCl + 2 mL HF
(C) 1 mL HNO_3 + 6 mL HCl + 1 mL HF
(D) 2 mL HNO_3 + 6 mL HCl + 2 mL HF
Sample 1: the ratio of 1 (shrimp sludge): 4 (crushed bark and husk): 0.5 animal manure (w/w).

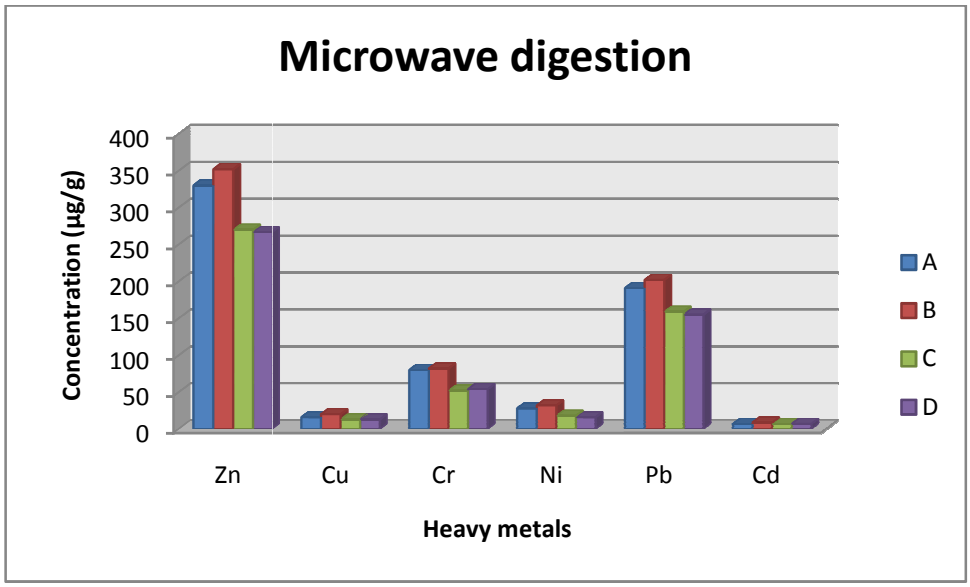


Figure 4.11: Effects of different solvent volume and systems digestion on elements concentration in sample 2 by microwave digestion
(A) 6 mL HNO_3 + 1 mL HCl + 1 mL HF
(B) 6 mL HNO_3 + 2 mL HCl + 2 mL HF
(C) 1 mL HNO_3 + 6 mL HCl + 1 mL HF
(D) 2 mL HNO_3 + 6 mL HCl + 2 mL HF
Sample 2: the ratio of 1 (compost shrimp sludge): 1 (coco peat waste)

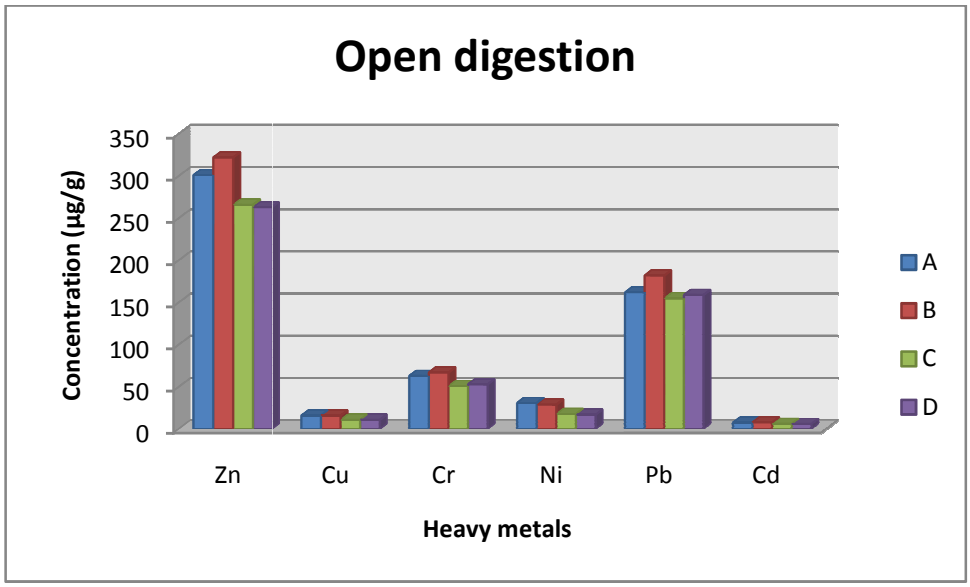


Figure 4.12: Effects of different solvent volume and systems digestion on elements concentration in sample 2 by microwave digestion
(A) 6 mL HNO_3 + 1 mL HCl + 1 mL HF
(B) 6 mL HNO_3 + 2 mL HCl + 2 mL HF
(C) 1 mL HNO_3 + 6 mL HCl + 1 mL HF
(D) 2 mL HNO_3 + 6 mL HCl + 2 mL HF
Sample 2: the ratio of 1 (compost shrimp sludge): 1 (coco peat waste)

4.2- Sungai Buloh and the straits of Melaka sediment samples

Many negative effects have been done on human health by the environmental pollution of heavy metals. The remediation of heavy metal pollution is often problematic due to their persistence and not degradability in the environment. As a sink and source, sediments constitute a reservoir of bioavailable trace elements and play an important role in geochemical cycles. Much concern has been focused on the investigation of the total metal contents in sediments. However, it cannot provide sufficient information about mobility, bioavailability and toxicity of metals. Their properties depend not only on their total concentration but also on the physicochemical form they occur which has been described as ‘‘speciation’’ (Ure *et al*., 1993).

Metals are distributed throughout sediment components and associated with them in various ways including ion exchange, adsorption, precipitation, and complexation. They are not permanently fixed by sediment. Changes in environment conditions such as acidification, redox potential or organic ligand concentrations may cause mobilization of element from solid to liquid phase and contamination of surrounding waters.

In this study, some samples from 7 stations in different depth were analyzed by BCR modified sequential extraction method (**Table 3.5**) to obtain the information of vertical and planar distributions of heavy metals in this region.

4.2.1. Variation of metal distribution patterns with depth

To investigate variation of distribution patterns of metals in the non-residual fractions with depth, seven sediment cores (with length between 0 and 45 cm) were collected at S1-S7.

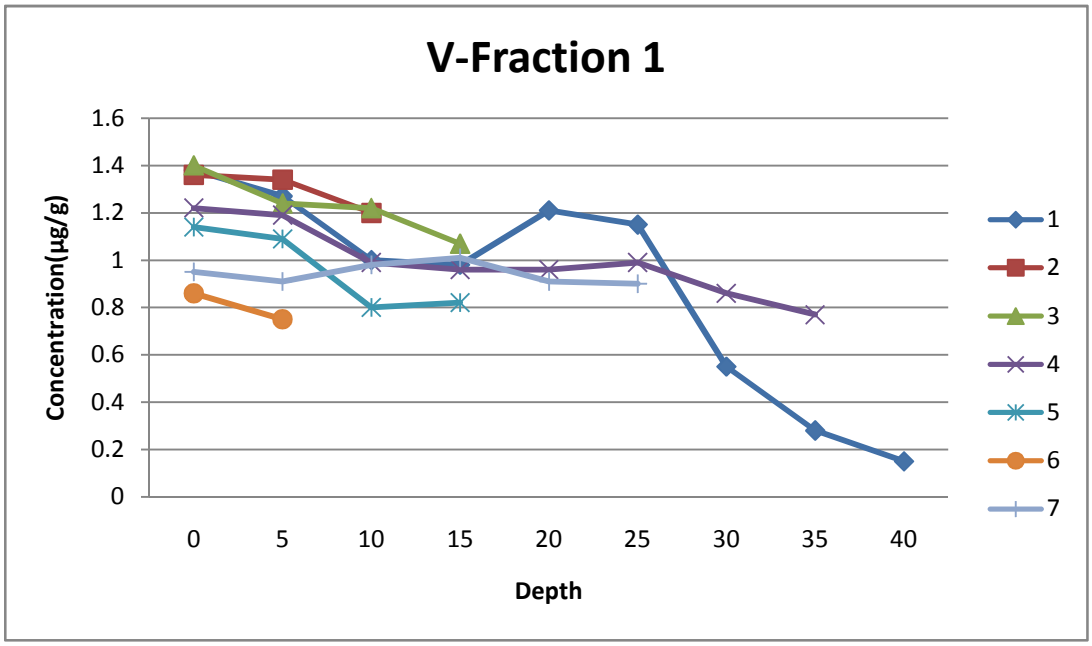
4.2.1.1- Fraction 1

The variation of element concentration in fraction 1 with depth at S1-S7 is shown in **Figures 4.13**. The concentration of metals in the top sediments were the highest compared to other depth subsamples for most of the elements at the seven sampling sites except for Cr at S7 in 15 cm depth and Ni at S3 that has a dominant increasing from top to down. Variation trends of concentration for each element in the seven sample cores were approximately similar. We can see a normal decreasing from top to down.

The surface enrichment may be due to contamination deposited from the surface waters, which also indicates that the pollutions are posed in the recent years. This is because the pollution is always absorbed into top sediment at first, and then sinks into more deep positions by chemical exchange.

The fact that much higher concentrations of V, Pb, Cd, Ni, Cu, Zn and Cr in the top sediments were found at S2 and S3 indicates that these two stations suffered more heavily pollution from these elements in other stations.

The discrimination became small with the deepening of depth, especially when the depth was deeper than 15 cm. This phenomenon may be interpreted by that the elements in the fraction A were mainly caused by pollution in the top sediments. With the deepening of depth, the effects of pollution become slighter and the distribution of elements mainly depends on the sediments themselves geographically.



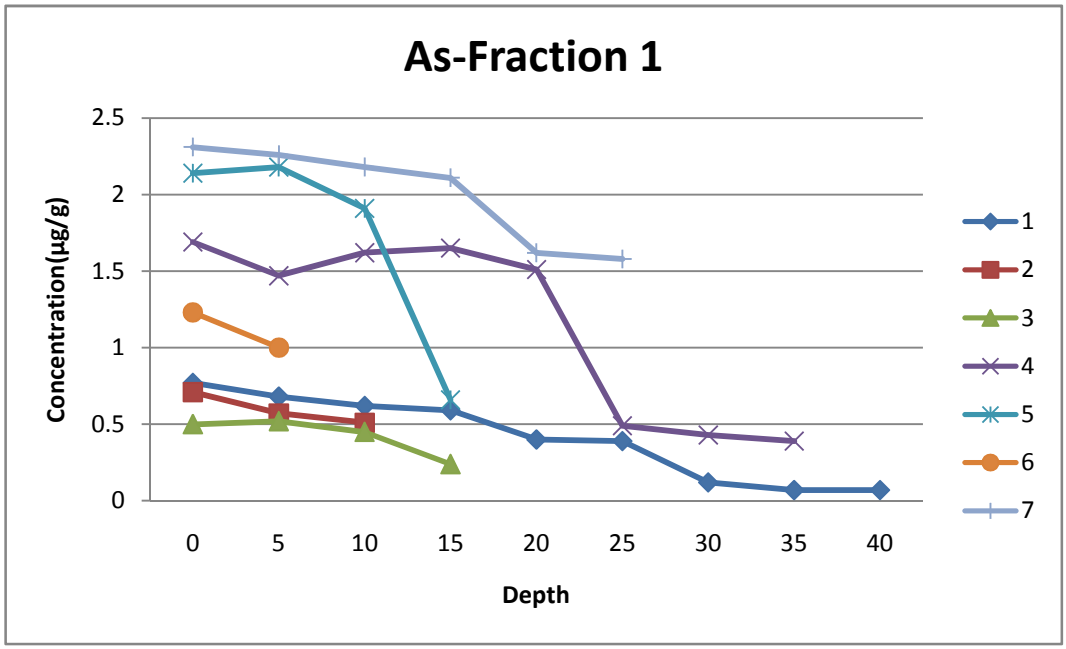
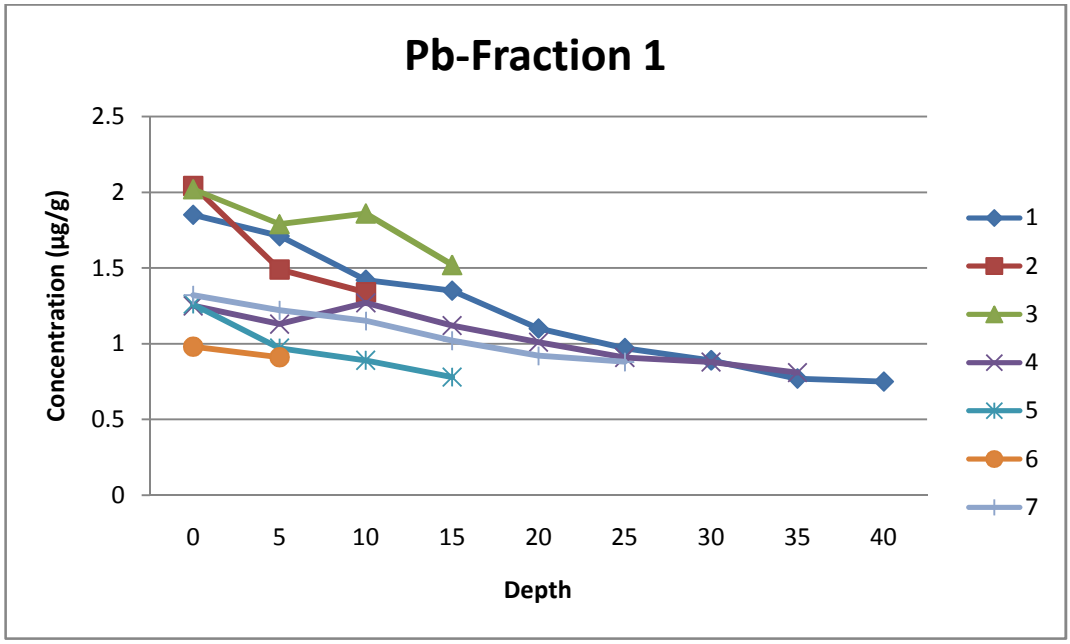
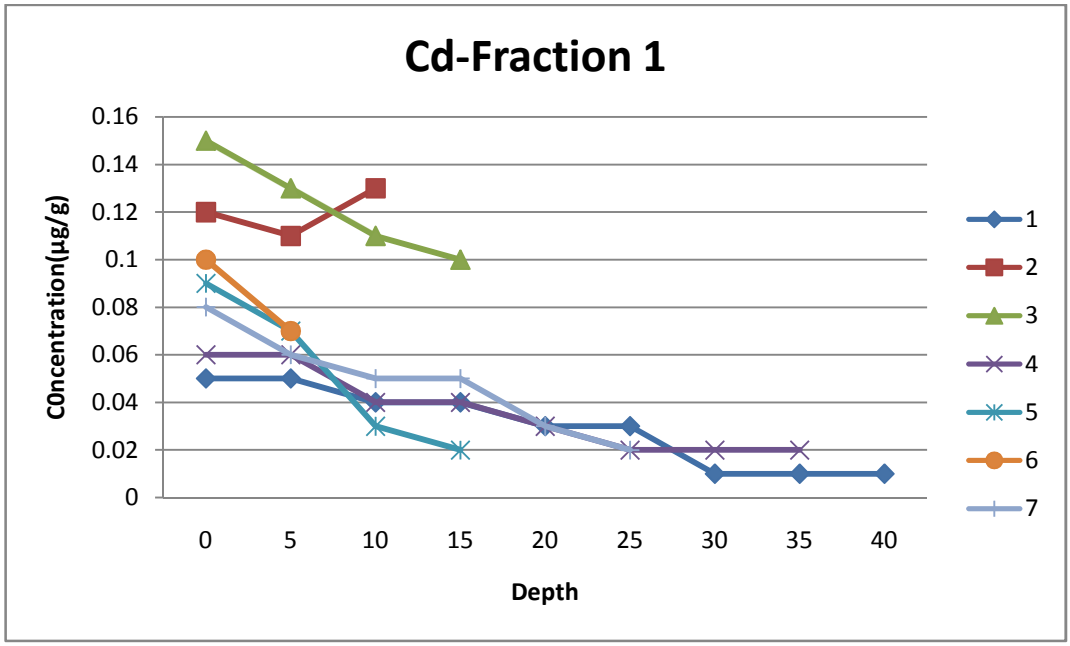
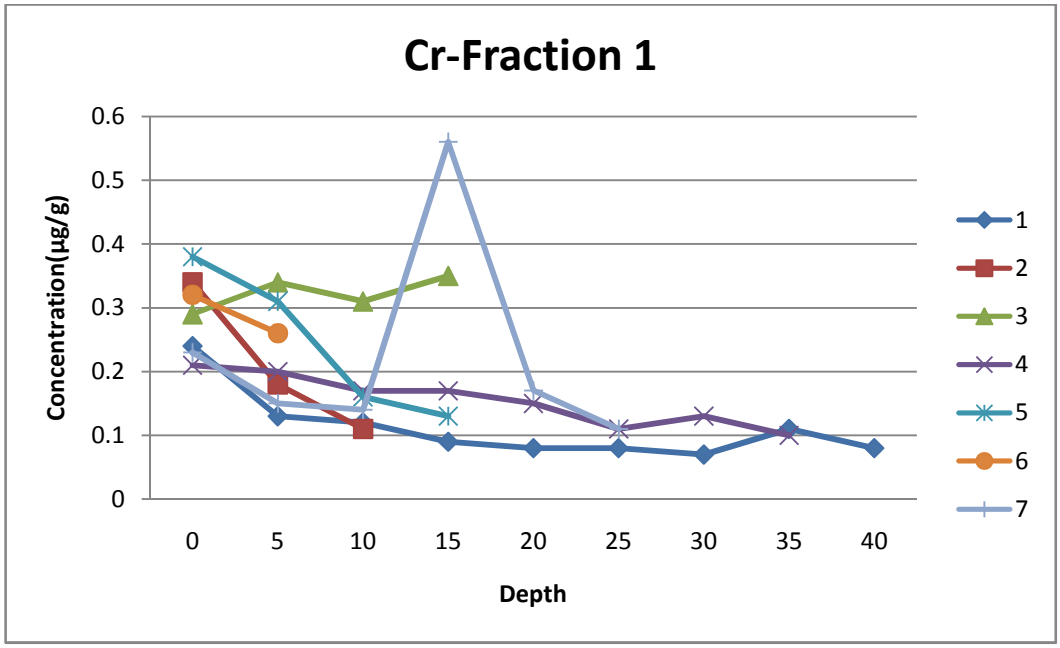


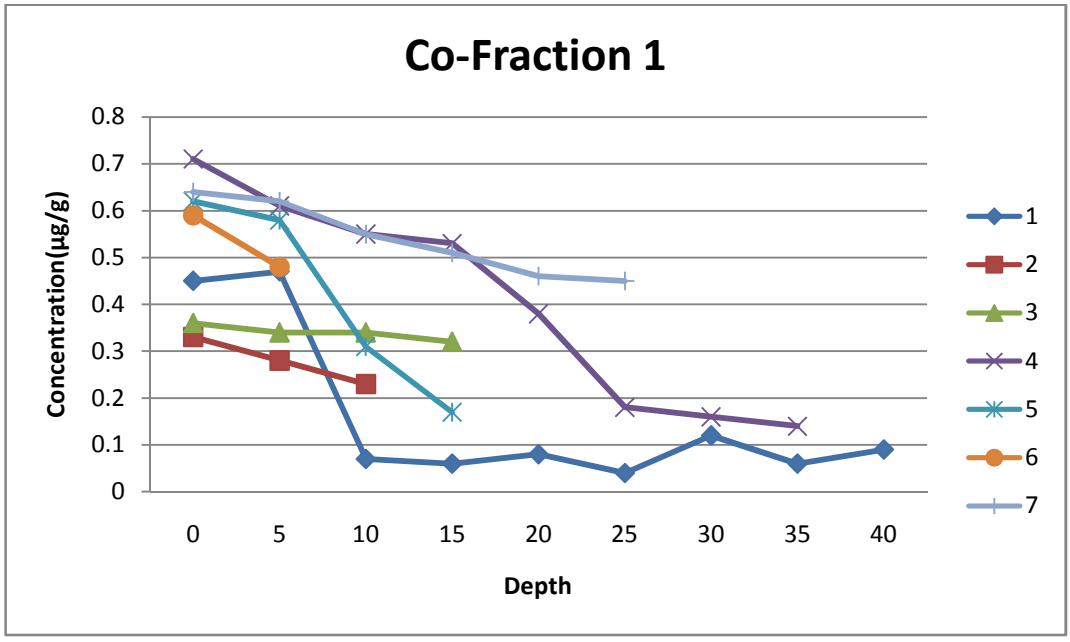
Figure 4.13: Concentration variation of elements in Fraction 1


Pb-Fraction 1
Concentration (µg/g)
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

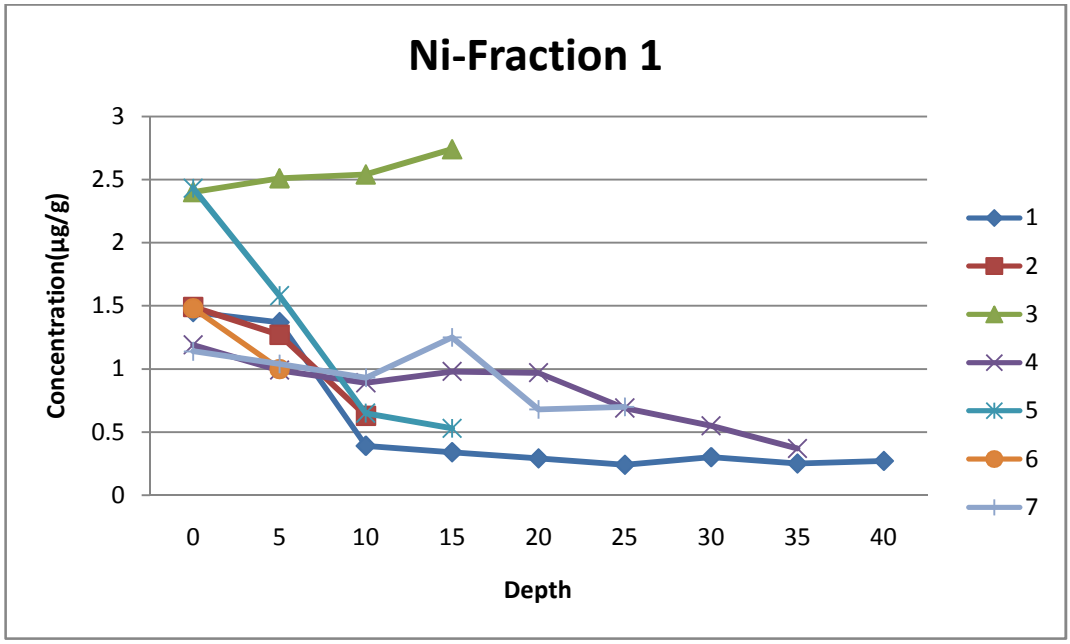

Cd-Fraction 1
COncentration(µg/g)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

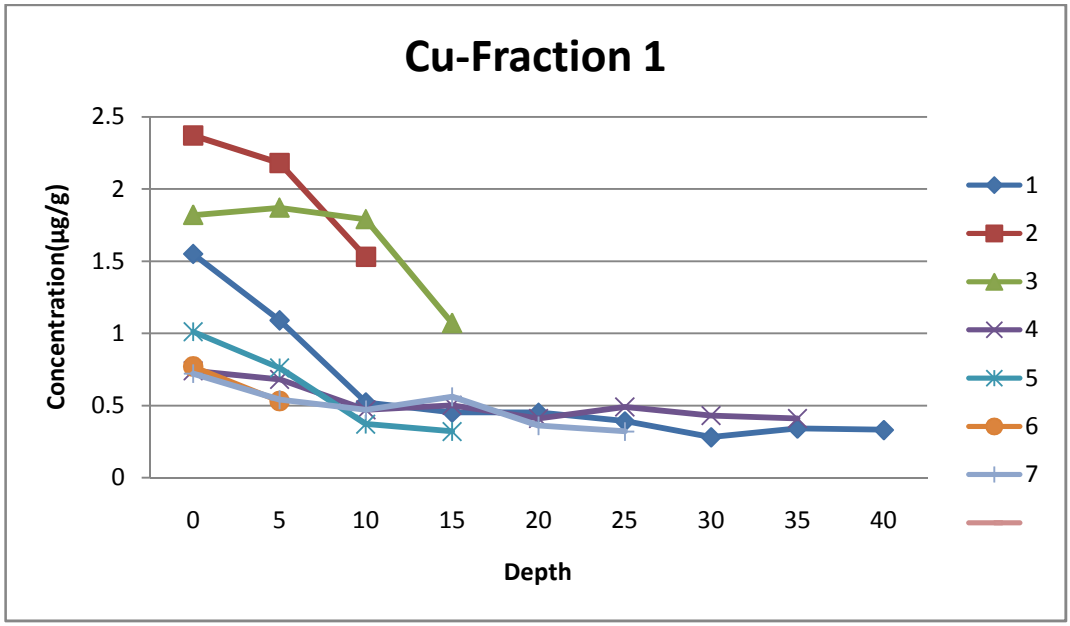
(Continued)


Cr-Fraction 1
Concentration(µg/g)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

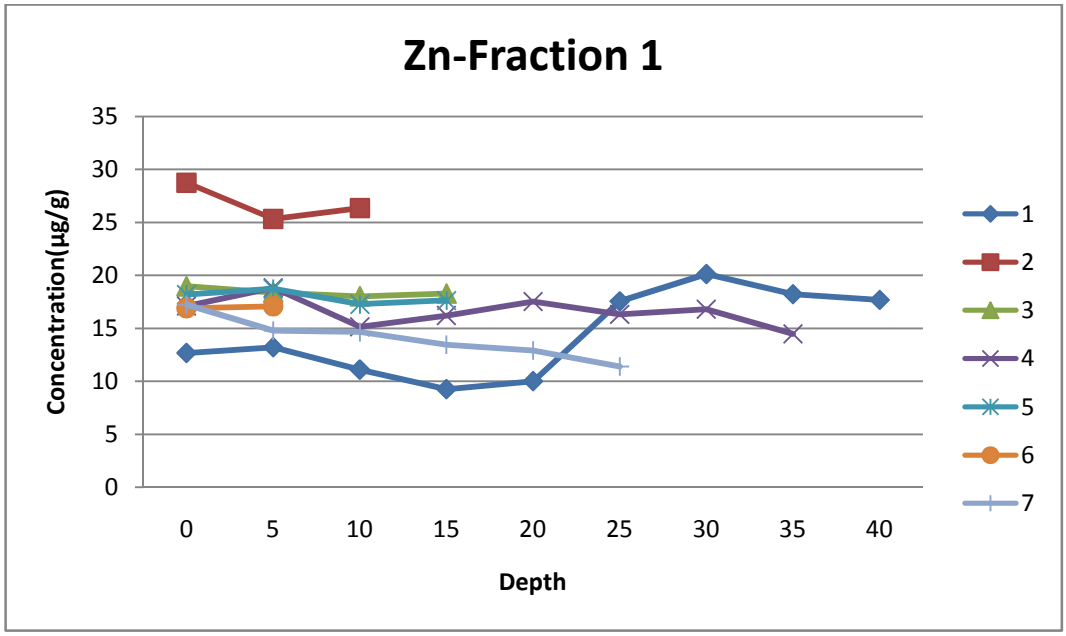

Co-Fraction 1
Concentration(µg/g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

(Continued)


Ni-Fraction 1
Concentration(µg/g)
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7


Cu-Fraction 1
Concentration(µg/g)
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

(Continued)

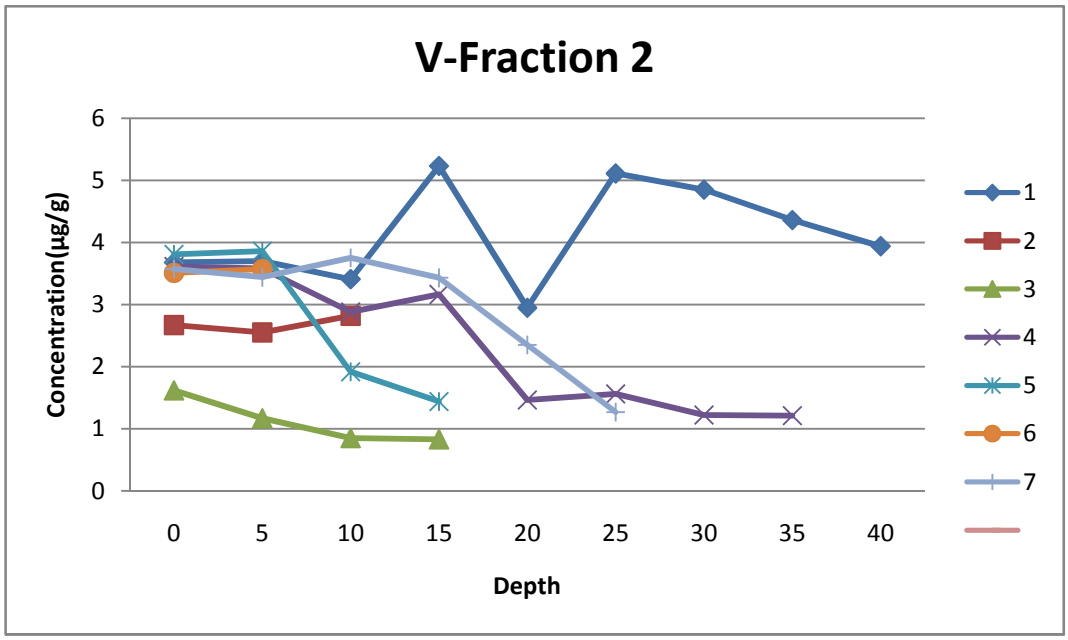

Zn-Fraction 1
Concentration(µg/g)
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

(Continued)

4.2.1.2- Fraction 2

The variation of element concentration in fraction 2 with depth at S1-S7 is shown in **Figure 4.14**. Higher concentrations were found in the top sediments for most of elements except for V at S1 and S4 and also As at S1, S3, S4, S5 and S7. It should be highlighted that much higher concentration of Pb, Cd, Cr, Ni and Zn have been seen at S2 and S3 in the top sediments in compare with other stations. Also Pb, Cd and Cu in Station 1 are investigable. We can see a exception at 15 cm depth for As at some stations and also Cr in Station 7 that it can be interpreted as a special contamination for As and Cr in a few years ago.

The results show that larger amount of elements may be sorbed by more Fe–Mn oxides in the top sediments than at the deeper positions. That could be interpreted by that the reductive dissolution of Fe–Mn oxides will occur at deeper position in the absence of significant sulphide which could fix the Fe and Mn as sulphide phase followed by precipitation upon crossing the oxic/anoxic boundary. The freshly formed Fe–Mn oxides may also scavenge a significant amount of upwards diffusing heavy metals. The discrimination became small with the deepening of depth for Zn and Ni.



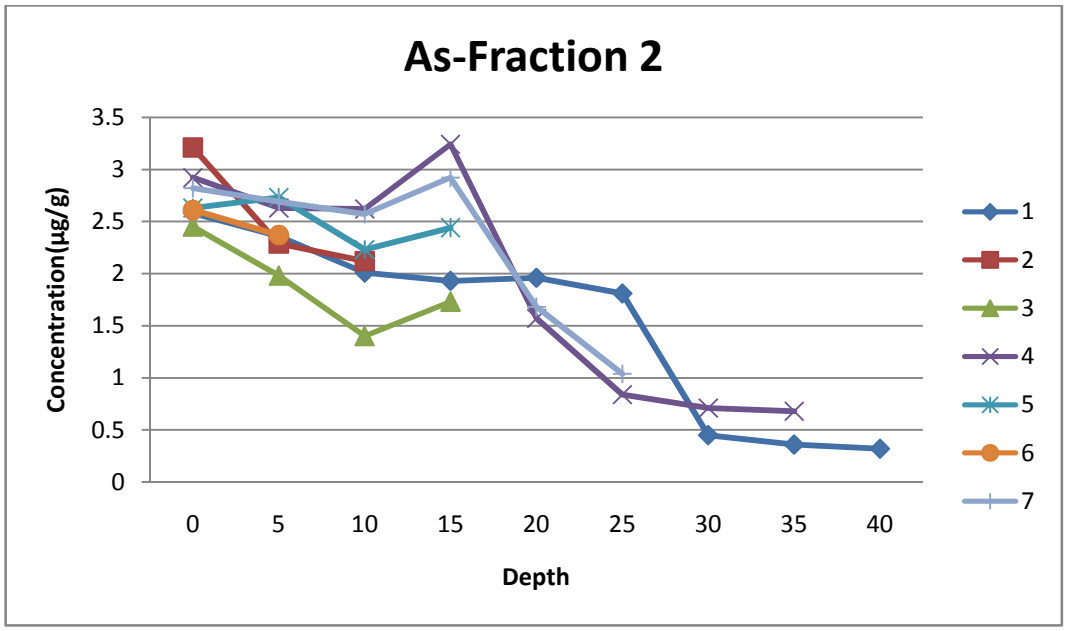
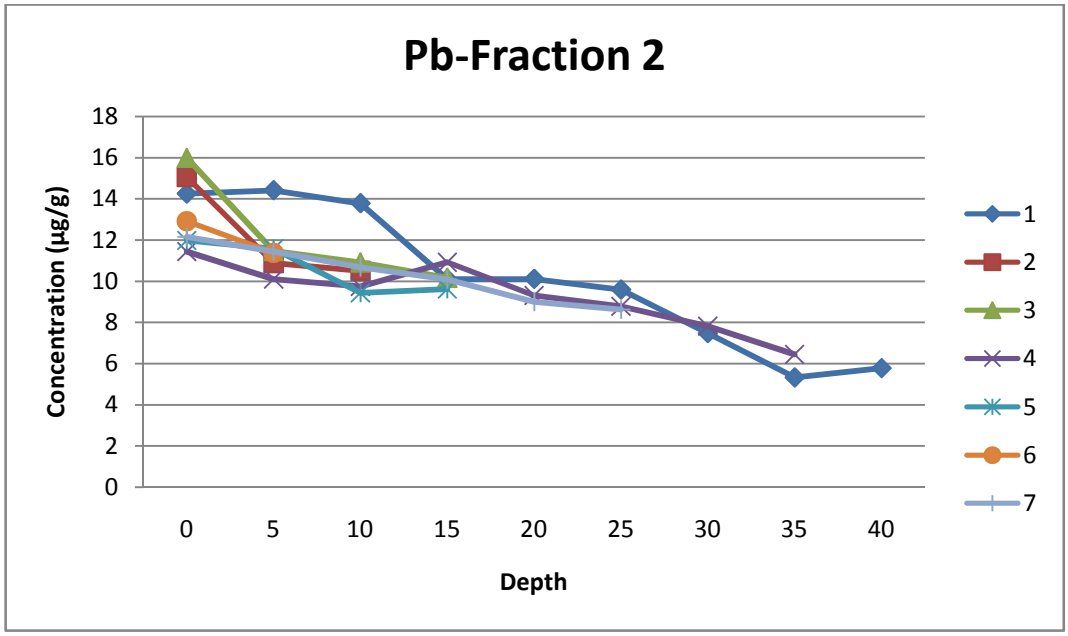
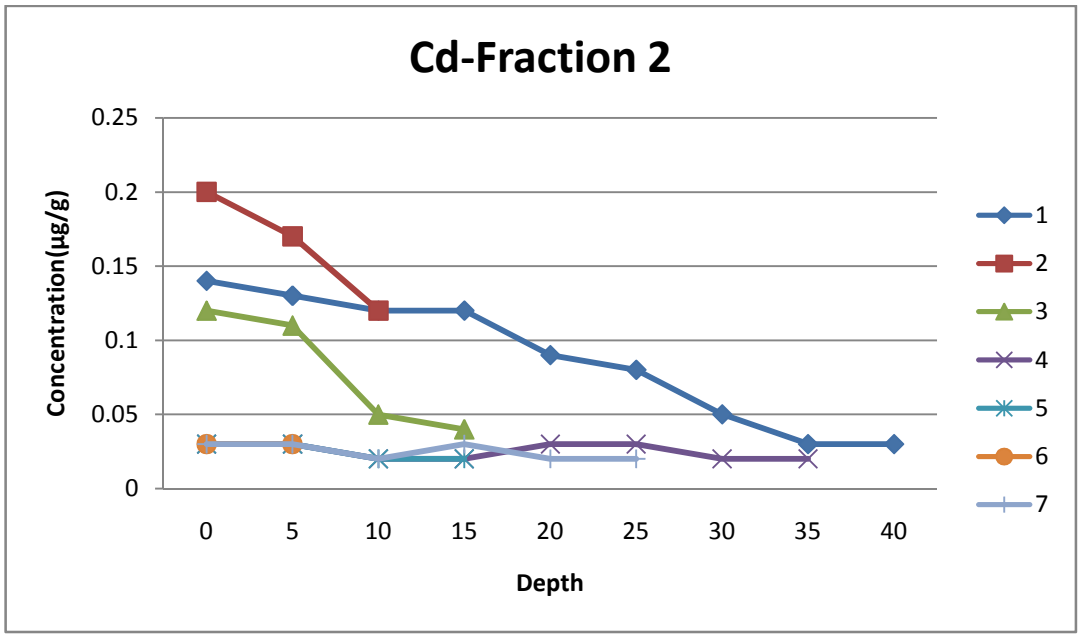
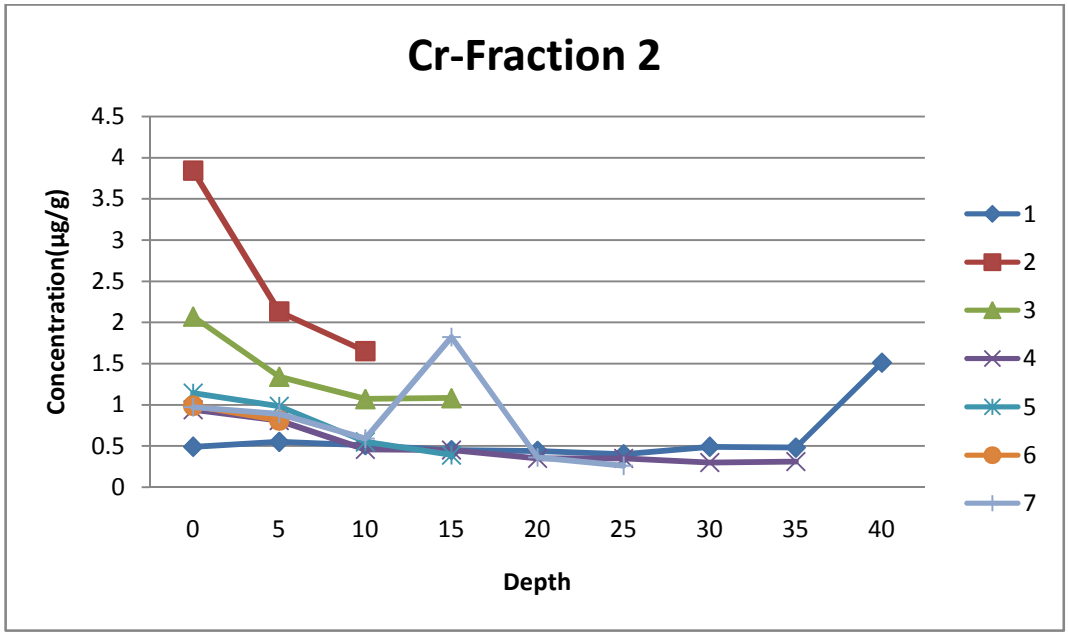


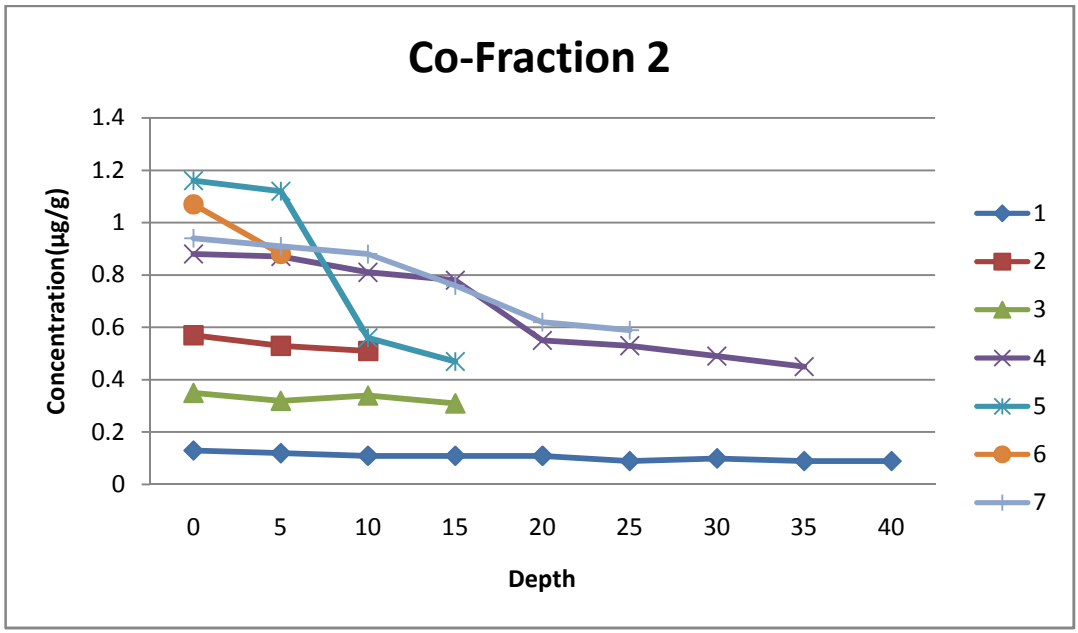
Figure 4.14: Concentration variation of elements in Fraction 2


Pb-Fraction 2
Concentration (µg/g)
18
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

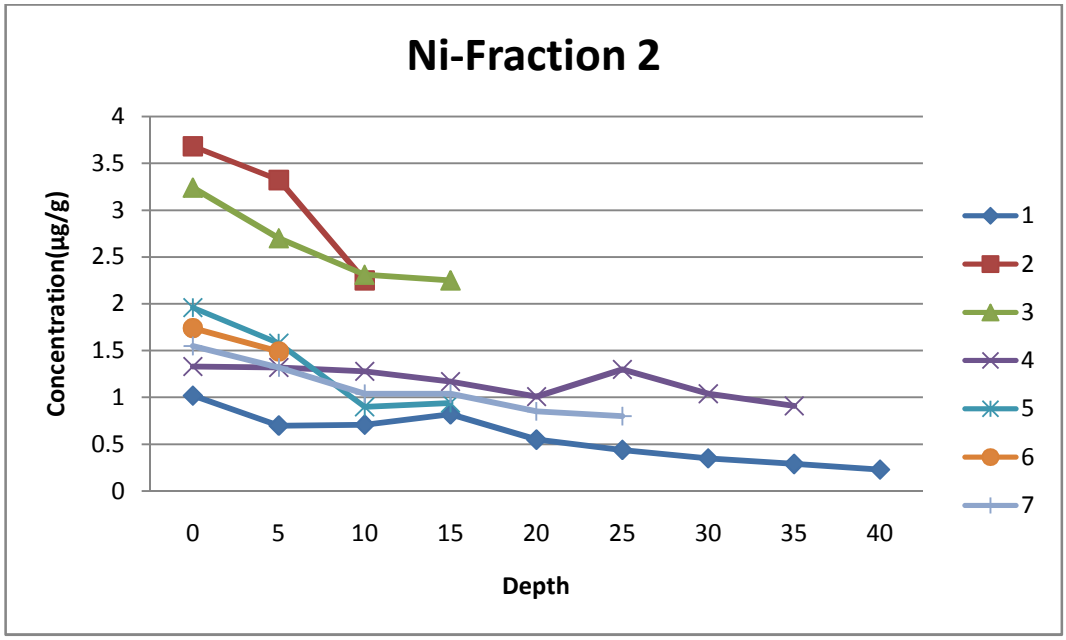

Cd-Fraction 2
Concentration(µg/g)
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

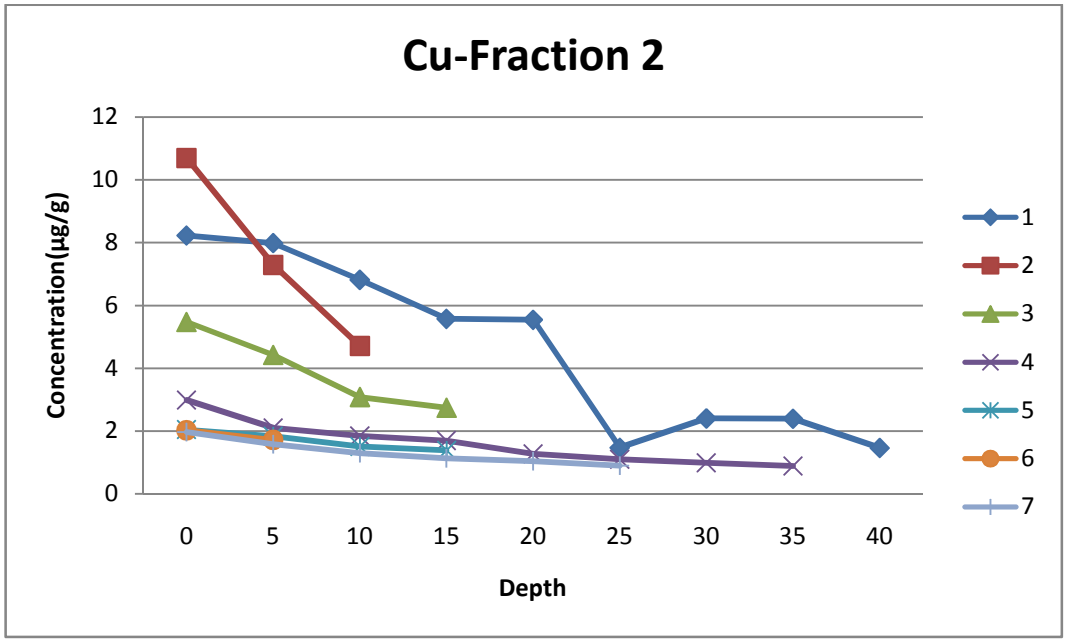
(Continued)


Cr-Fraction 2
Concentration(µg/g)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

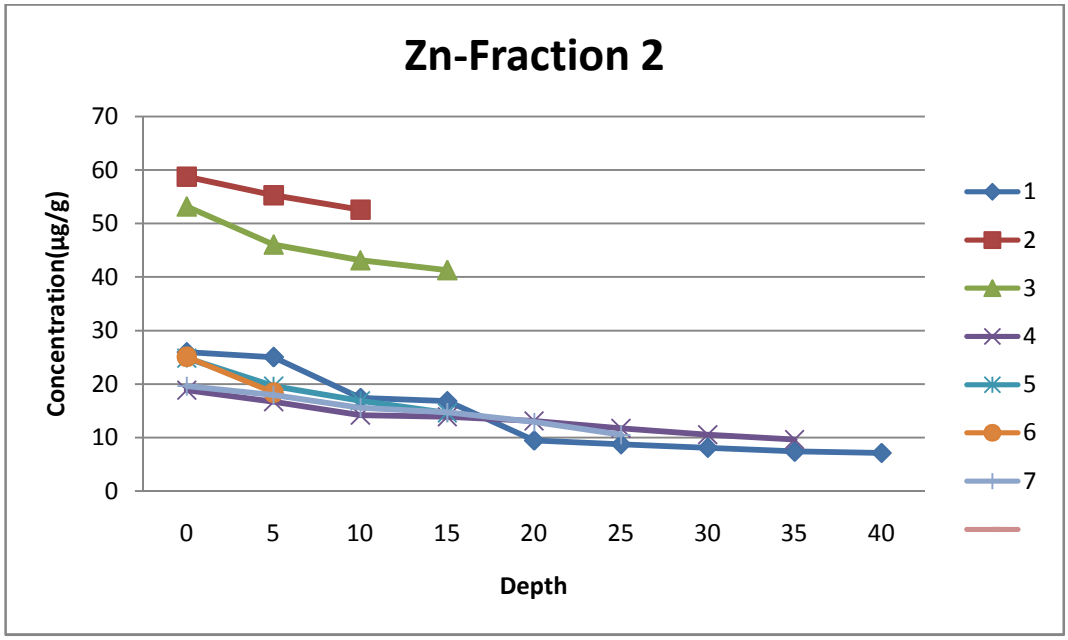

Co-Fraction 2
Concentration(µg/g)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

(Continued)


Ni-Fraction 2
Concentration(µg/g)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7


Cu-Fraction 2
Concentration(µg/g)
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

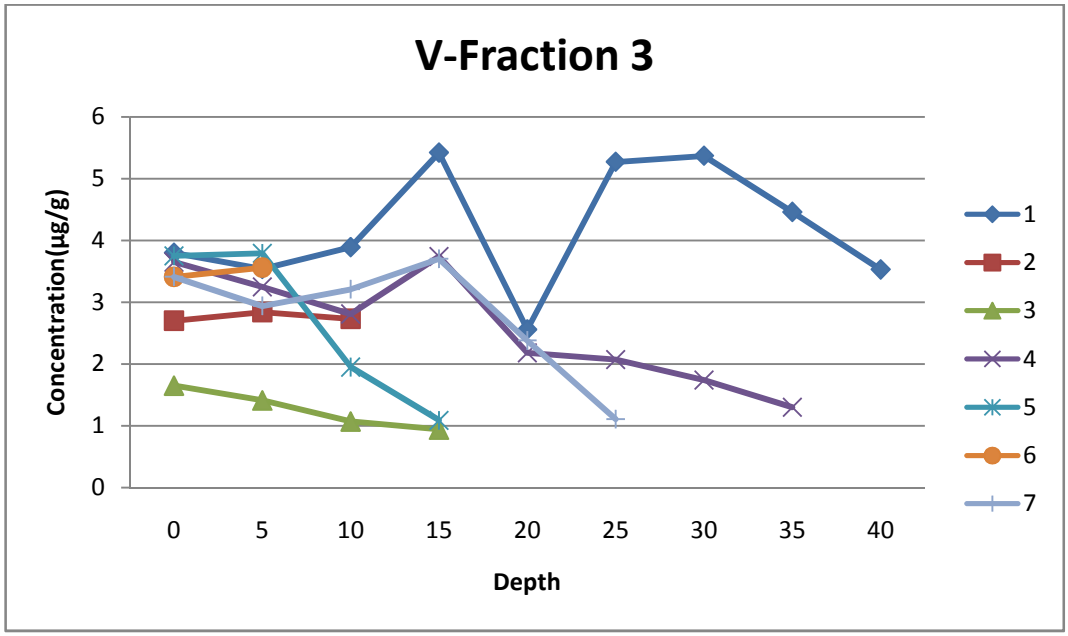
(Continued)


Zn-Fraction 2
Concentration(µg/g)
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

(Continued)

4.2.1.3- Fraction 3

The variation of element concentration in fraction 3 with depth at S1-S7 is shown in **Figure 4.15**. The variation of the concentrations of elements in this fraction was more complex than the two fractions. There was no evident rule that could be concluded from the results, but some useful information could be obtained. The concentration of all elements except V, As and Co in station 2 and 3 are higher than other stations. Also we can see these result in station 1 for Pb, Cd and Cu. Elements in the fraction 3 mainly bound to various forms of organic matter by complexation and of natural organic matter or bioaccumulation in certain living organisms through different ways. So the variation of the elements in this fraction became more complex and irregular than that in the fractions 1 and 2.



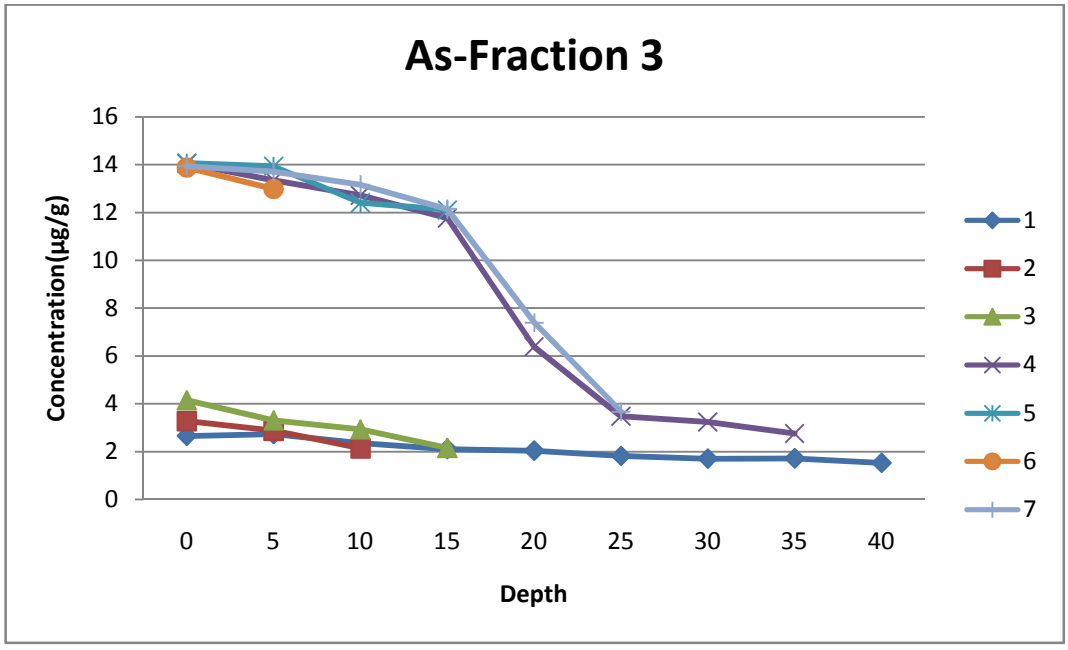
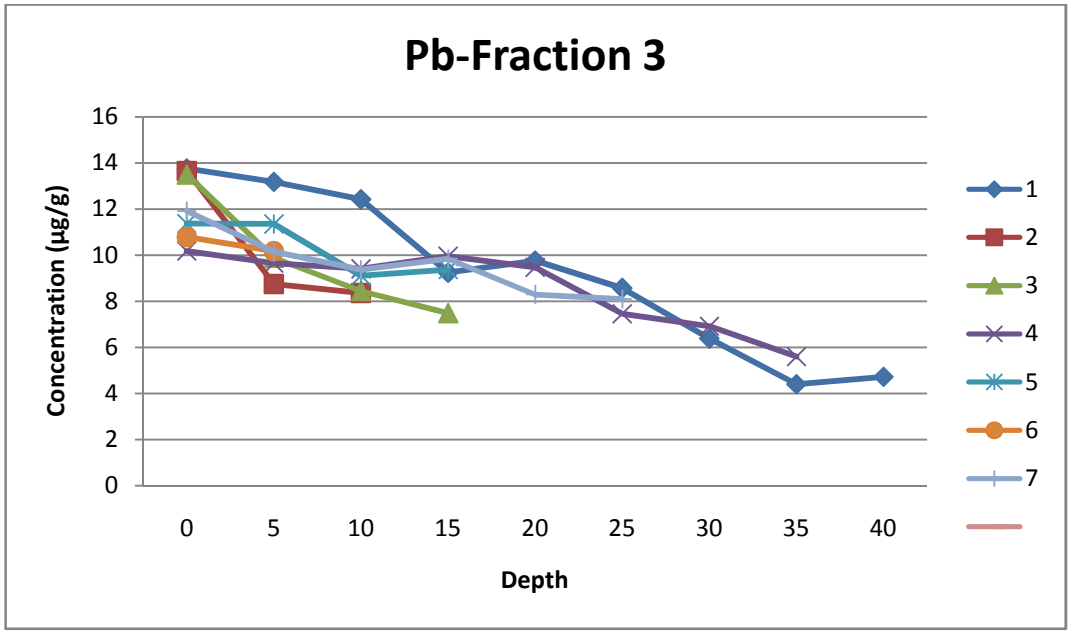
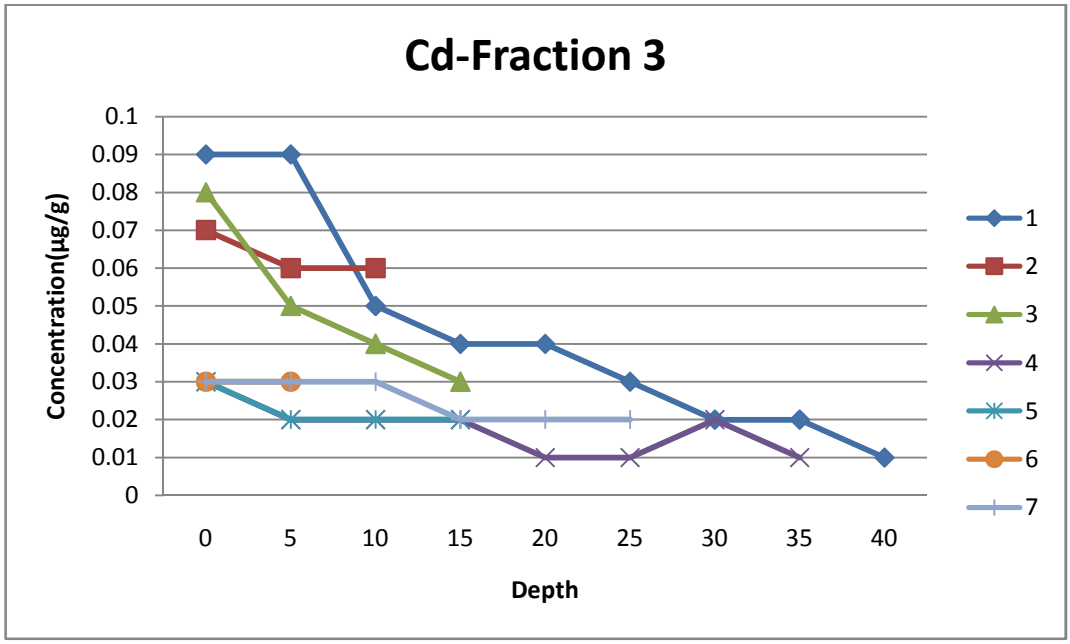
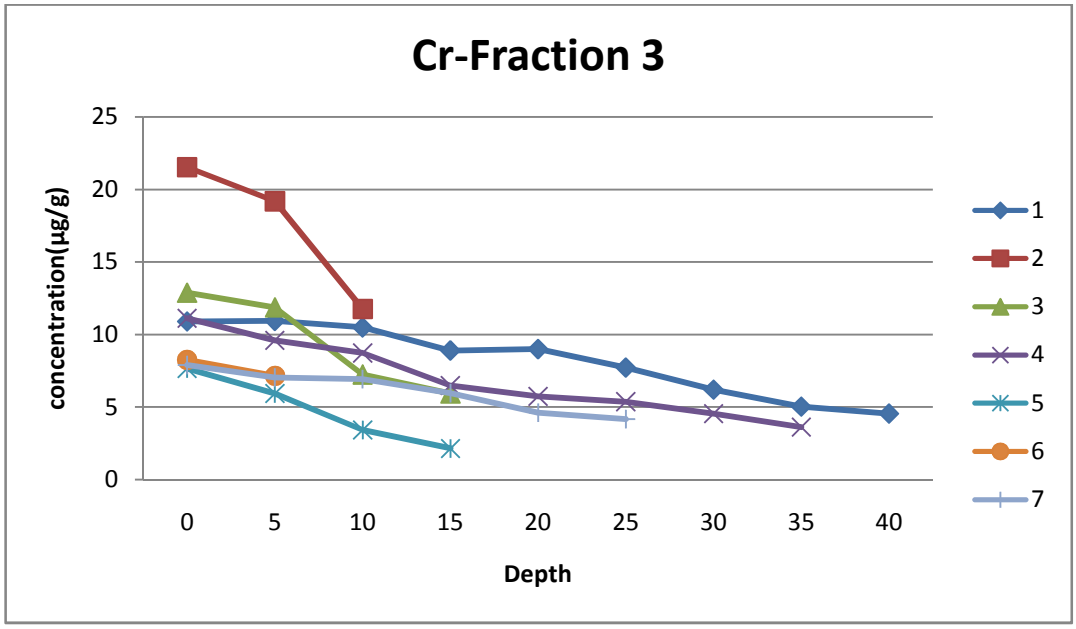


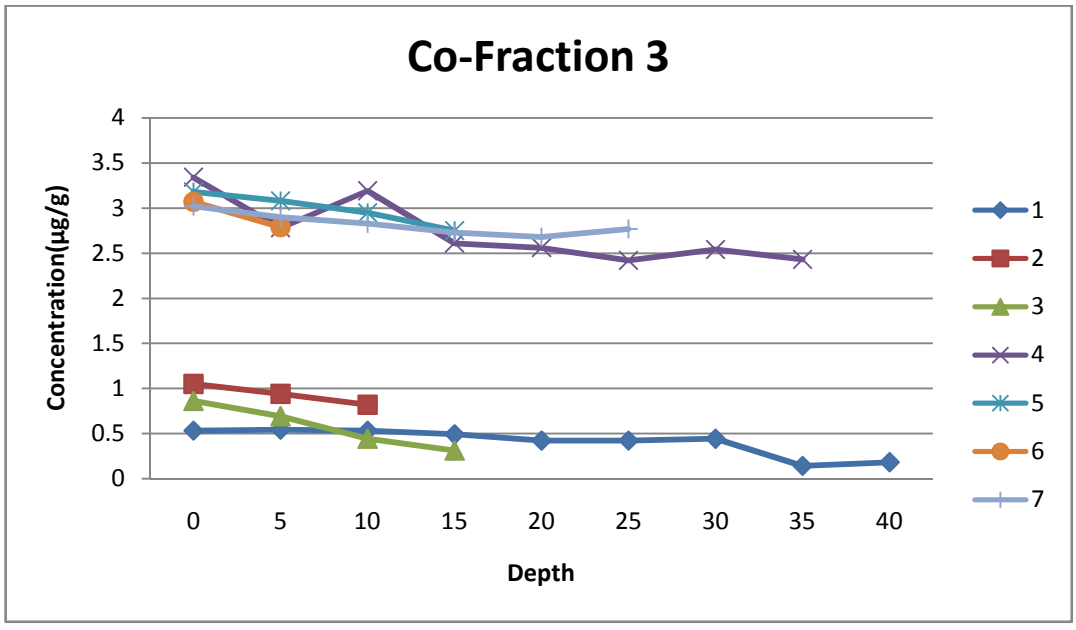
Figure 4.15: Concentration variation of elements in Fraction 3


Pb-Fraction 3
Concentration (µg/g)
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

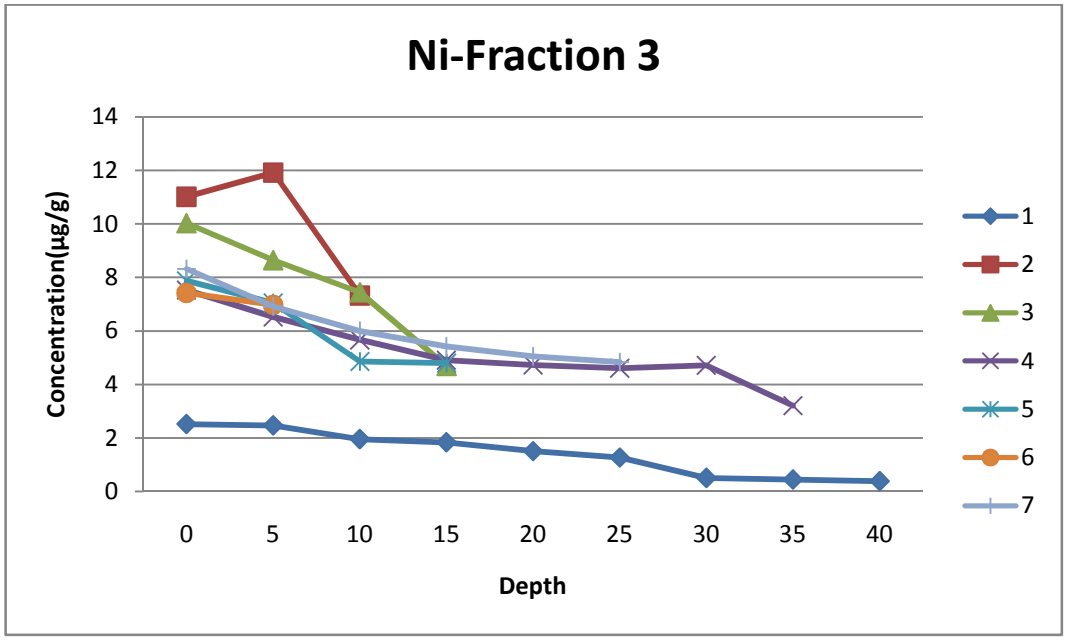

Cd-Fraction 3
Concentration(µg/g)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

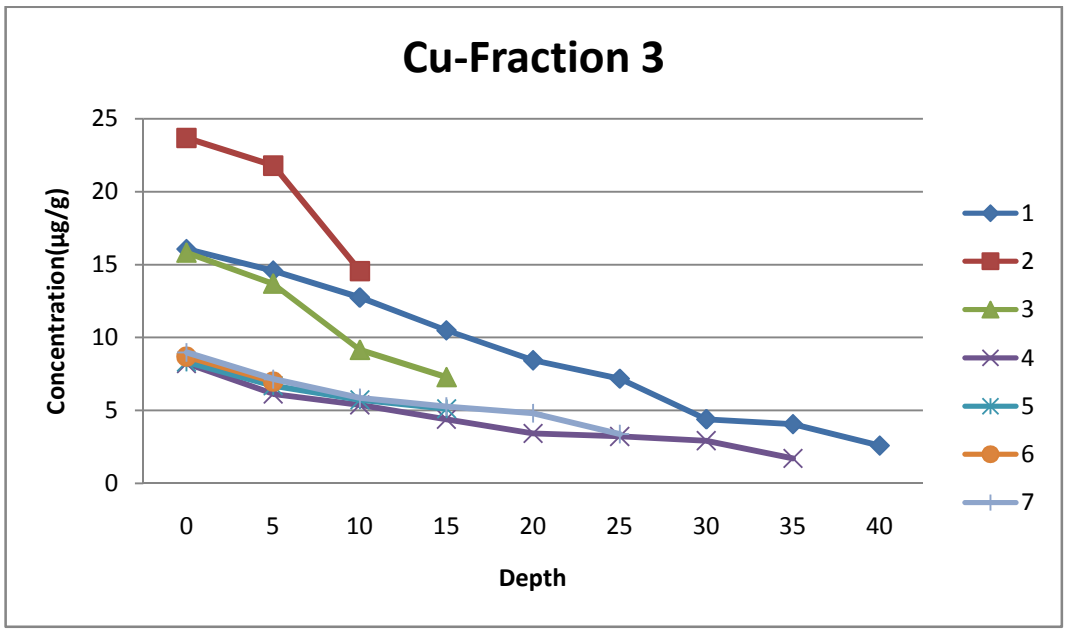
(Continued)


Cr-Fraction 3
concentration(µg/g)
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

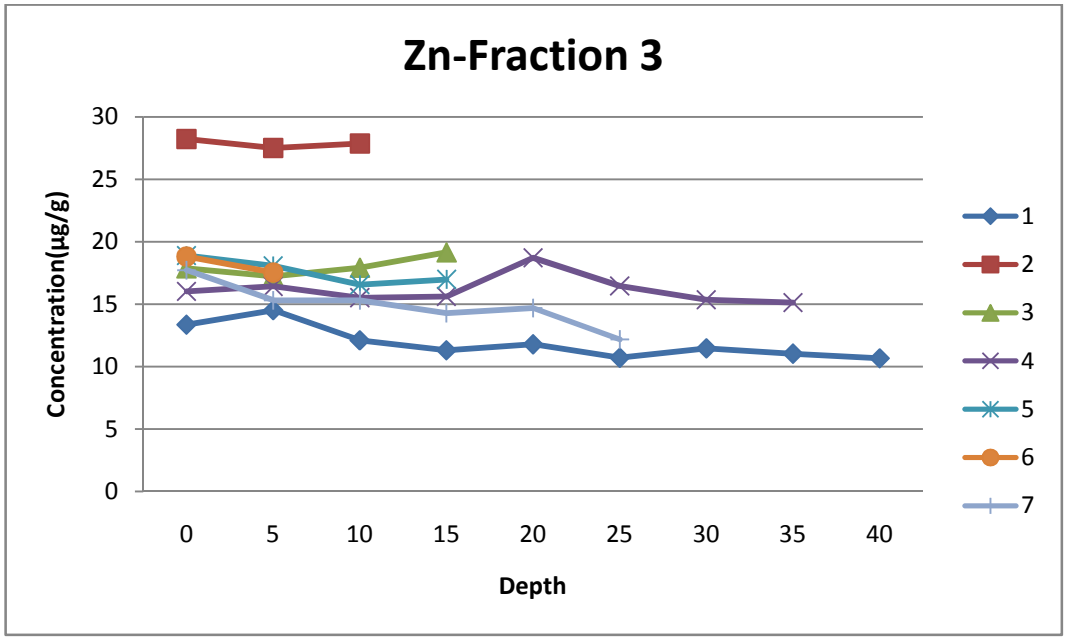

Co-Fraction 3
Concentration(µg/g)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

(Continued)


Ni-Fraction 3
Concentration(µg/g)
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7


Cu-Fraction 3
Concentration(µg/g)
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

(Continued)


Zn-Fraction 3
Concentration(µg/g)
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
Depth
1
2
3
4
5
6
7

(Continued)

4.2 2. Sequential extraction results in the surface sediments

The metal concentrations in surface sediments of the river and marine sediment samples from each extraction step are shown in **Table 4.14**. The discussion on the distribution patterns of elements is divided into five groups depending on the degree of their association with the different phases. V and Cr were found to be present mainly in the residual fraction, while Co and Ni were found in the oxidizable fraction. In addition, Zn and Pb were found in a group with the large proportion of the total concentration presenting in the easily reducible fraction, also Cd was found in the acid extractable/exchangeable fraction, moreover, large amounts of Cu were obtained in oxidizable fraction at Stations 1-3 and residual fraction from Stations 4-7.

4.2.2.1. Vanadium and Chromium

In this study, the results show that V and Cr are mainly found in the residual fraction (**Figure 4.16)**, representing 73.9-86.52% and 52.78-75.44% respectively. The phase distribution of V and Cr in this study is similar to the results reported by Yuan et al. [16], indicating that V and Cr were mostly retained in the residual fraction. Elements associated with the residual fraction are likely to be incorporated in aluminosilicate minerals, and so are unlikely to be released to pore-waters through dissociation. Less than 10%, 10% and 5% of V have been found in oxidisable, easily reducible and exchangeable fractions, respectively. The highest amount of V was obtained at S3 in residual fraction. However, the amount of Cr in Fractions 1, 2 and 3 was only less than 1%, 8% and 40% respectively at all stations.

Furthermore, Cr found at S5 showed the highest portion in residual fraction. In this case, the portion of oxidisable fraction is noticeable too.

Table 4.14: Concentration of metals by modified BCR SEP (µg g-1), N=3

	V	Cr	Co	Ni	Cu	Zn	Cd	Pb
Station No. 1								
Fraction 1	1.38±0.07*	0.24±0.03	0.45±0.05	1.45±0.09	1.55±0.06	18.58±0.92	0.05±0.00	1.85±0.08
Fraction 2	3.68±0.13	0.49±0.03	0.13±0.01	1.02±0.07	8.22±0.16	25.99±1.51	0.14±0.01	14.25±1.61
Fraction 3	3.80±0.11	10.89±0.86	0.53±0.05	2.52±0.10	16.06±0.41	13.36±0.77	0.09±0.00	13.76±1.49
Residue	25.11±2.23	25.67±2.91	0.51±0.05	4.38±0.17	12.67±0.28	12.67±0.71	0.05±0.0	9.71±1.73
Three step + Residue	33.97±2.54	37.29±3.83	1.62±0.16	9.37±0.43	38.50±0.91	70.60±3.91	0.33±0.01	39.57±4.91
Pseudo-total	34.17±3.31	37.83±3.61	1.59±0.13	9.55±0.51	39.14±2.03	68.75±4.86	0.35±0.01	40.14±3.88
Recovery (%)	99.41	98.57	101.88	98.11	98.36	102.69	94.28	98.58
C_f	0.35	0.45	2.17	1.14	2.03	4.57	5.60	3.07
RAC (%)	4.06	0.64	27.77	15.47	4.02	26.31	15.15	4.67
Station No. 2								
Fraction 1	1.36±0.06	0.34±0.05	0.33±0.05	1.49±0.07	2.37±0.16	36.96±3.70	0.12±0.01	2.04±0.02
Fraction 2	2.67±0.17	3.84±0.25	0.57±0.07	3.68±0.14	10.69±0.58	58.74±5.33	0.20±0.03	15.06±0.09
Fraction 3	2.70±0.13	21.52±2.62	1.05±0.03	11.00±0.66	23.67±1.90	28.22±2.46	0.07±0.00	13.65±1.04
Residue	24.4±2.28	28.73±2.55	0.38±0.03	6.23±0.49	18.73±1.73	28.73±1.99	0.13±0.00	12.62±1.04
Three step + Residue	31.13±2.64	54.43±5.47	2.33±0.18	22.40±1.36	55.46±4.37	152.65±13.4	0.52±0.04	43.37±2.19
Pseudo-total	31.88±3.03	55.04±5.62	2.28±0.24	22.26±1.77	55.96±4.92	157.31±11.6	0.55±0.06	43.66±2.00
Recovery (%)	97.64	98.89	102.19	100.62	99.10	97.03	94.54	99.33
C_f	0.27	0.89	5.13	2.59	1.96	4.31	2.69	2.43
RAC (%)	4.36	0.62	14.16	6.65	4.27	24.21	23.07	4.70
Station No. 3								
Fraction 1	1.40±0.04	0.29±0.01	0.36±0.07	2.40±0.07	1.82±0.06	44.03±3.22	0.15±0.02	2.02±0.03
Fraction 2	1.62±0.04	2.07±0.06	0.35±0.07	3.24±0.11	5.47±0.38	53.16±2.77	0.12±0.00	15.99±0.77
Fraction 3	1.65±0.03	12.87±0.18	0.86±0.16	10.02±0.41	15.81±2.07	17.86±0.69	0.08±0.01	13.49±0.48
Residue	29.99±2.88	23.39±0.79	0.26±0.02	4.88±0.22	8.96±1.29	18.96±1.15	0.10±0.00	10.21±0.51
Three step + Residue	34.66±2.99	38.62±1.04	1.83±0.32	20.54±0.81	32.06±3.80	134.01±7.83	0.45±0.03	41.71±1.79
Pseudo-total	35.21±3.21	38.17±2.64	1.91±0.25	21.02±1.67	32.61±2.88	137.29±5.82	0.48±.05	42.10±1.62
Recovery (%)	98.43	101.17	95.81	97.71	98.31	97.61	93.75	99.07
C_f	0.15	0.65	6.03	3.21	2.57	6.06	3.50	3.08
RAC (%)	4.04	0.75	19.67	11.68	5.67	32.85	33.33	4.84
Station No. 4								
Fraction 1	1.22±0.03	0.21±0.05	0.71±0.09	1.19±0.14	0.74±0.08	13.16±2.17	0.06±0.00	1.25±0.02
Fraction 2	3.61±0.28	0.94±0.93	0.88±0.09	1.33±0.09	2.98±0.38	18.86±2.02	0.03±0.00	11.44±0.17
Fraction 3	3.65±0.15	11.11±1.51	3.34±0.36	7.53±0.62	8.20±0.61	16.03±1.74	0.03±0.00	10.17±0.08
Residue	36.30±3.92	27.10±3.89	0.68±0.06	5.42±0.48	17.10±1.90	17.11±2.05	0.06±0.00	9.15±0.08
Three step + Residue	44.78±4.38	39.36±6.38	5.61±0.60	15.47±1.33	29.02±2.97	65.16±7.98	0.18±0.00	32.01±0.35
Pseudo-total	45.27±5.62	39.77±4.62	5.59±0.73	15.83±1.49	29.66±3.33	65.48±5.82	0.19±0.01	32.49±2.73
Recovery (%)	98.91	98.96	100.35	97.72	97.84	99.49	94.73	98.52
C_f	0.23	0.45	7.24	1.85	0.69	2.80	2.00	2.49
RAC (%)	2.72	3.74	12.65	7.69	2.55	20.19	33.33	3.90
Station No. 5								
Fraction 1	1.14±0.12	0.38±0.04	0.62±0.09	2.43±0.31	1.01±0.78	17.74±2.27	0.09±0.00	1.26±0.16
Fraction 2	3.81±0.17	1.14±0.17	1.16±0.11	1.96±0.16	2.04±0.27	24.82±2.11	0.03±0.00	11.98±0.94
Fraction 3	3.75±0.14	7.65±0.61	3.18±0.26	7.87±0.92	8.31±0.63	18.90±1.59	0.03±0.00	11.37±1.32
Residue	36.73±0.54	28.17±2.62	0.64±0.05	5.47±0.48	18.17±0.20	18.17±1.34	0.08±0.00	9.27±1.00
Three step + Residue	45.43±0.97	37.34±3.44	5.60±0.51	17.73±1.87	29.53±1.88	79.63±7.31	0.23±0.00	33.88±3.42
Pseudo-total	45.75±1.37	37.90±2.89	5.93±0.74	17.42±1.66	29.88±2.70	80.19±6.29	0.23±0.01	33.62±3.10
Recovery (%)	99.30	98.52	94.43	101.78	98.82	99.30	100.00	100.77
C_f	0.23	0.32	7.75	2.24	0.62	3.38	1.87	2.65
RAC (%)	2.51	1.01	11.07	13.70	3.42	22.27	39.13	3.71
Station No. 6								
Fraction 1	0.86±0.06	0.32±0.03	0.59±0.07	1.48±0.11	0.77±0.04	17.33±1.49	0.10±0.01	0.98±0.12
Fraction 2	3.51±0.28	0.99±0.08	1.07±0.11	1.74±0.11	2.02±0.17	25.10±2.00	0.03±0.00	12.91±1.30
Fraction 3	3.41±0.36	8.24±0.66	3.07±0.39	7.42±0.59	8.69±1.15	18.82±1.38	0.03±0.00	10.78±1.17
Residue	35.44±4.19	25.89±1.72	0.56±0.08	5.37±0.53	15.89±1.48	16.89±1.64	0.06±0.00	8.89±0.07
Three step + Residue	43.22±4.89	35.44±2.39	5.29±0.65	16.01±1.24	27.37±2.84	78.14±6.51	0.22±0.01	33.56±2.66
Pseudo-total	43.64±4.30	35.41±3.07	5.55±0.52	15.89±1.42	27.52±2.13	79.01±7.08	0.23±0.03	33.97±3.13
Recovery (%)	99.03	100.08	95.31	100.75	99.45	98.89	95.65	98.79
C_f	0.22	0.36	8.44	1.98	0.72	3.62	2.66	2.77
RAC (%)	1.98	0.90	11.15	9.24	2.81	22.17	45.45	2.92
Station No. 7								
Fraction 1	0.95±0.08	0.23±0.04	0.64±0.04	1.14±0.09	0.72±0.04	14.52±1.81	0.08±0.00	1.32±0.11
Fraction 2	3.57±0.29	0.97±0.08	0.94±0.11	1.55±0.13	1.96±0.22	19.61±2.60	0.03±0.00	12.15±1.40
Fraction 3	3.41±0.32	7.89±0.95	3.02±0.27	8.31±0.68	8.97±0.71	17.71±1.36	0.03±0.00	11.91±1.25
Residue	38.27±2.83	23.21±1.61	0.61±0.04	5.21±0.42	17.21±2.08	17.21±1.72	0.05±0.00	9.11±0.82
Three step + Residue	46.20±3.52	32.3±2.68	5.21±0.46	16.21±1.32	28.86±2.95	69.05±7.49	0.19±0.00	34.49±3.58
Pseudo-total	46.74±4.50	32.44±1.93	5.19±5.67	16.97±1.51	28.37±2.36	69.80±6.66	0.18±0.02	34.92±
Recovery (%)	98.84	99.56	100.38	95.52	101.72	98.92	105.55	98.76
C_f	0.20	0.39	7.54	2.11	0.67	3.01	2.80	2.78
RAC (%)	2.05	0.71	12.28	7.03	2.49	21.02	42.10	3.82

***Mean±SD**

4.2.2.2. Cobalt and Nickel

The distribution patterns of Co and Ni are illustrated in **Figure 4.16**. Co and Ni were distributed in all four fractions of the sediment phases, but the dominant portion was obtained in the oxidisable fraction. The dominant phase in the oxidisable fraction accounted for more than 56% of the total concentration of elements at stations S4-S7 and less than 50% at stations S1-S3. There was a portion between 10% - 20% in exchangeable and residual fraction at all stations except Co at station S1. The considerable proportion of total Ni concentration (about 50%) was in the oxidisable fraction at all stations except S1 that was found in residual fraction. However, Ni showed less than 17% of total Ni in Fractions 1 and 2 at all stations.

4.2.2.3. Zinc and Lead

The dominant portion of Zn and Pb were in easily reducible fraction (iron-manganese oxide fraction). Zn showed a variation between 28% and 40% in Fraction 2 while a noticeable amount of this element was observed in exchangeable fraction at S1-S3. Although the highest concentration of Pb was obtained in Fraction 2, very similar results were found for Fractions 1 and 3. Less Pb content was obtained in exchangeable fraction (>5%).

4.2.2.4. Cadmium

The levels of Cd in sediments and sludge have been the focus of much concern for a long time due to its high toxicity. Several sequential extraction procedures, including the BCR protocol have been used to obtain information on the distribution of Cd in sediment [17-19]. Not only sediments, but also other samples such as soil [20, 21] and fly-ash samples [22], have been determined for the concentrations of Cd phase

distribution by sequential extraction methods. In this study, Cd was also detected in the marine sediments following the BCR sequential extraction. The results of sequential extraction for Cd are illustrated in **Figure 4.16**.

As we can see, the variation of Cd was more significant than other elements. For example, more than 33% of the total Cd concentration was observed in exchangeable fraction at stations S3-S7 while less than 23% was obtained at stations S1 and S2. The highest concentrations of total Cd were found in easily reducible fraction at stations S1 and S2. Noticeable portion of Cd was found in residual fraction at stations S4-S7.

4.2.2.5. Copper

Cu showed exactly two different patterns in river and marine sediments. The high portion of Cu was obtained in oxidisable fraction at stations S1-S3 (Sungai Buloh River sediments) and residual fraction at stations S4-S7 (the Straits of Malacca sediments). About 30% of total Cu was found at S1-S3 in residual fraction and oxidisable fraction at stations S4-S7.

4.2.3. Pseudo total metal digestion

The results of Pseudototal metal digestion are presented in **Table 4.14** and the amounts of heavy metals found at seven sampling stations are compared in **Table 4.15**.

The highest total amounts of elements after pseudototal metal digestion were obtained for Cr, Ni, Cu, Zn, Cd and Pb at station S2. This is due to the sediment sample was collected exactly from outside a metal factory. It showed that the production of metallic tube industries play an important role in river pollution. Also, high concentrations of V and Co were obtained in samples taken from marine locations. As we know, one of the major sources of V and Co are oil and petrol products. Therefore, these sample matrices have been contaminated by shipping industries, fishing industries or boating which are

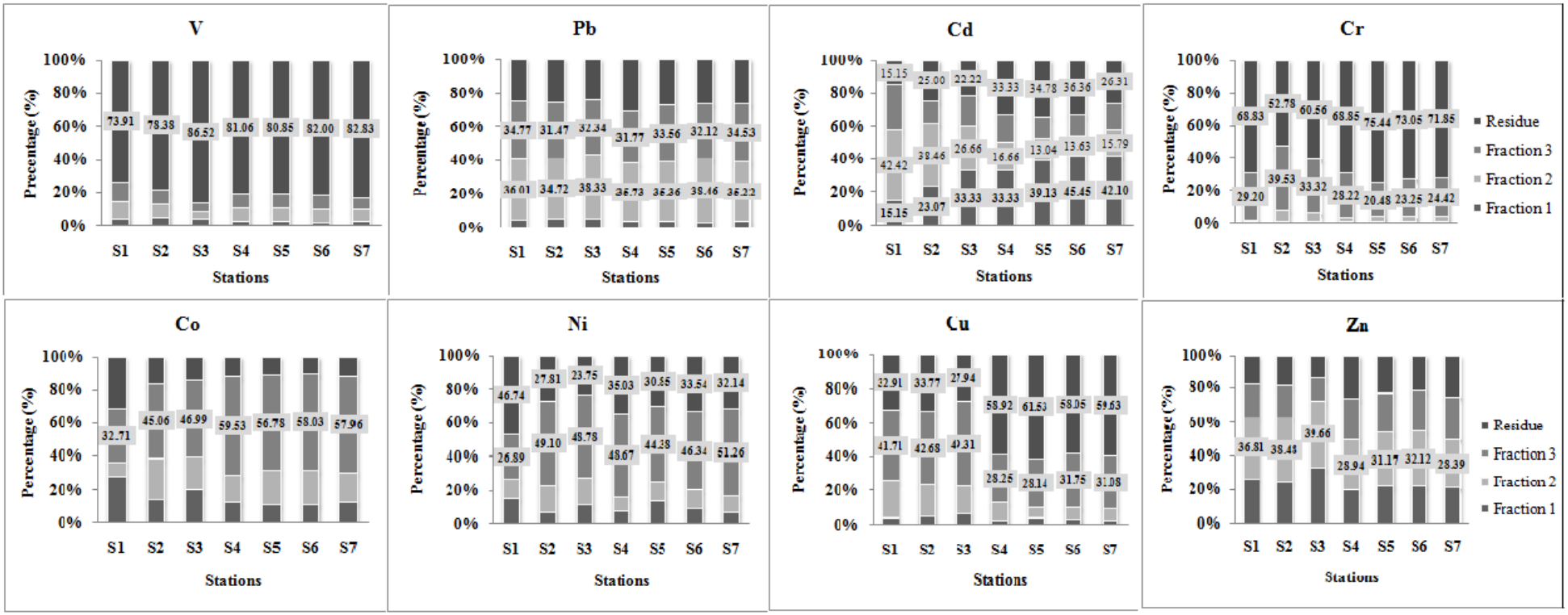


Figure 4.16: The average percentage of element speciation in different stations using modified BCR SEP method

the common anthropogenic activities in this area. The contamination in Sungai Buloh sediments increased from upstream to downstream. Nevertheless, high element contamination was obtained at station S2 due to the metal industry. In addition, much of the municipal wastewater is discharged into this river, thus increasing river pollution.

Table 4.15: Comparison of total element digestion using pseudo total metal digestion method

Elements	Concentrations in total digestion
V	S7>S5>S4>S6>S3>S1>S2
Cr	S2>S4>S3>S5>S1>S6>S7
Co	S5>S4>S6>S7>S2>S3>S1
Ni	S2>S3>S5>S7>S6>S4>S1
Cu	S2>S1>S3>S5>S4>S7>S6
Zn	S2>S3>S5>S6>S7>S1>S4
Cd	S2>S3>S1>S5>S6>S4>S7
Pb	S2>S3>S1>S7>S6>S5>S4

4.2.4. Internal check recovery

An internal check was performed on the results of the sequential extraction by comparing the total amount of metal extracted by different reagents during the sequential extraction procedure with the results of the total digestion. The recovery of the sequential extraction method was calculated as follows:

$$\text{Recovery} = [C_{\text{Fraction 1}} + C_{\text{Fraction 2}} + C_{\text{Fraction 3}} + C_{\text{Residue}} / C_{\text{total digestion}}] \times 100$$

The results shown in **Table 4.14** indicate that the sums of the four fractions are in good agreement with the total digestion results, with satisfactory recoveries (94.43-105.55 %) and the method used is reliable and repeatable.

4.2.5. Environmental implications

To study heavy-metal retention in sediment samples, the individual contamination factors (Cf) of elements and Risk assessment Code (RAC) in Sungai Buloh and Selat Melaka samples were calculated (**Table 4.14**).

4.2.5.1. Contamination factor (C_f)

The determination of heavy-metal contamination factor is an important aspect that indicates the degree of heavy metals risk to the environment in relation with its retention time. A high contamination factor of heavy metals shows low retention time and high risk to the environment. The individual contamination factor (C_f) of heavy metals was used to estimate the relative retention time of heavy metals retained in the sediment. It is determined by dividing the sum of each heavy metal concentration in the mobile phase (non-residue phase) by its concentration in the residual phase.

Figure 4.17 show the estimated contamination factors of each metal in the surface samples at all stations. The calculated factors in both types of sediment show the highest C_f and the ability of Cd, Co, Zn and Pb to be released from Sungai Buloh (S1-S3),

whereas V and Cr show the lowest. The residual concentration of any heavy metal is considered a non-mobile fraction and is an important part in influencing the mobility nature of the heavy metal. The combined effect of Cd, Zn, and Pb in high concentrations and with high mobility potential shows the increased possible risk of these metals to the environment (S1-S3).

The highest contamination factor was obtained for Co, Zn and Pb in the sediment samples obtained from the Straits of Malacca sediments (S4-S7), while the lowest was found for V and Cr, similar to Sungai Buloh sediments.

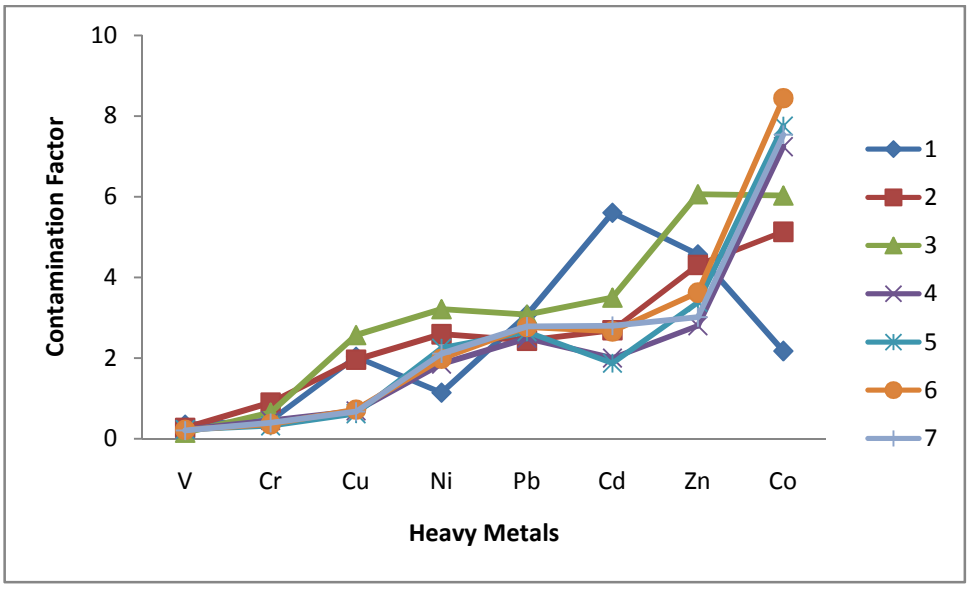


Figure 4.17: Estimated contamination factor of each metal in the surface samples at 7 stations

4.2.5.2. Risk Assessment Code (RAC)

The risk assessment code, defined as the fraction of metal exchangeable and/or associated with carbonates (% F1 for BCR), was determined for the eight trace metals, and the values interpreted in accordance with the RAC classifications. This classification is described by Perin et al. [23].

Metals are bound to different sediment fractions, with the binding strength determining their bioavailability and the risk associated with their presence in aquatic systems. The risk assessment code (RAC) was determined based on the percentage of the total metal content that was found in the first sediment fraction in BCR method (% F1). This indicates that the metals are weakly bound to the solid phase. Hence, the metals pose a greater risk to the aquatic environment due to their greater potential [24]. When this percentage mobility is less than 1%, the sediment has no risk to the aquatic environment. Percentages of 1–10% reflect low risk, 11–30% medium risk, and 31–50% high risk. Above 50%, the sediment poses a very high risk and is considered dangerous, with metals easily able to enter the food chain [23, 24].

Table 4.14 shows the results of RAC with values given as percentage of the fraction soluble in acid and carbonate fraction (% F1).

In general, the sediments show low risk for V, Cr, Cu and Pb with RAC values less than 10%, so, there is not any significant metal mobility for these elements. A medium risk is indicated for Co, Zn (except at S3), Cd at S1 and S2 and Ni at S1, S3 and S5 that it can be noticeable in the early future. Zn at S3 and Cd at S3-S7 show high risk for our sediment samples. Therefore, a significant remediation must be applied for Zn and Cd mobilization as soon as possible. From the selected samples there are no elements at very high risk conditions. **Table 4.16** shows the comparison of RAC values for all stations and elements.

Table 4.16: Comparison of RAC values for all stations and elements

	V	Cr	Co	Ni	Cu	Zn	Cd	Pb
S1	L	L	M	M	L	M	M	L
S2	L	L	M	L	L	M	M	L
S3	L	L	M	M	L	H	H	L
S4	L	L	M	L	L	M	H	L
S5	L	L	M	M	L	M	H	L
S6	L	L	M	L	L	M	H	L
S7	L	L	M	L	L	M	H	L

4.3- Speciation of heavy metals in sediment by modified BCR, ultrasound and microwave assisted sequential extraction procedure on CRM BCR 701 in different time

The comparison between modified BCR method, ultrasound method and microwave assisted digestion method for CRM-BCR 701 has been resulted in **Table 4.17**. Also the recovery between each method and certified sample has been mentioned.

The lowest recoveries were found for USE and highest recoveries were obtained for BCR modified method while the result of MSE also was noticeable.

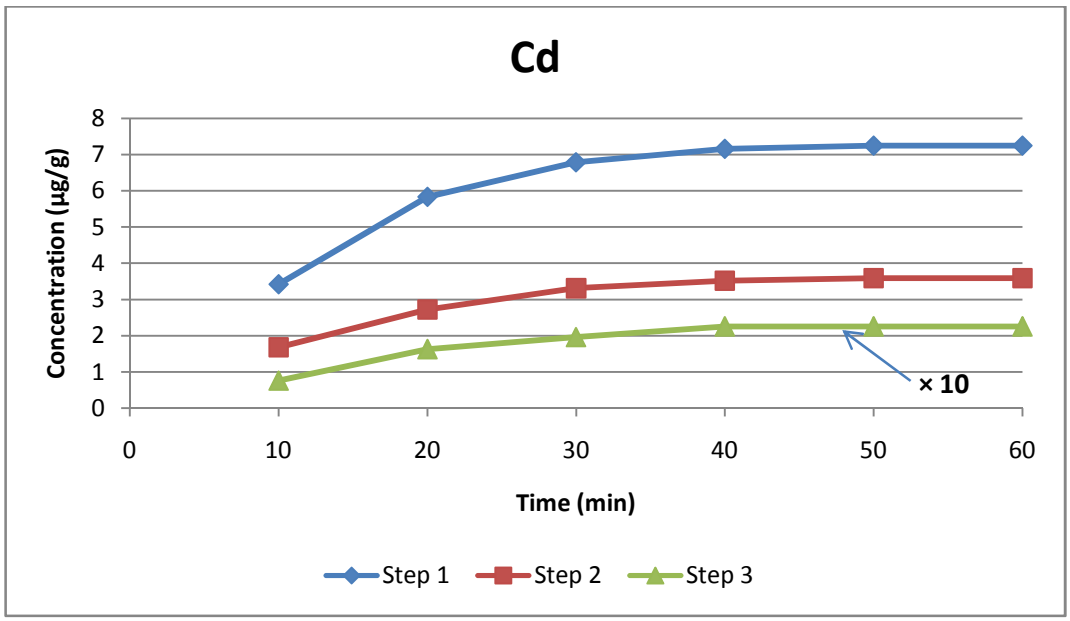
Table 4.17: Comparison between modified BCR, Ultrasonic and microwave assisted digestion method for CRM 70, N=3

	Technique	Cd	Cr	Cu	Ni	Pb	Zn
				Fraction 1			
Certified Values	Modified BCR	7.34±0.35*	2.26±0.16	49.3±1.7	15.4±0.9	3.18±0.21	205.0±6.0
This work	SEM	7.32±0.11	2.24±0.24	49.02±2.8	15.22±1.1	3.13±0.17	202.8±5.2
		99.72	99.11	99.43	98.83	98.42	98.92
	USE	7.25±0.42	2.18±0.18	47.91±1.2	14.77±0.7	3.05±0.11	202.8±7.4
		98.77	96.46	97.18	95.90	95.91	98.92
	MSE	7.30±0.16	2.22±0.25	48.83±1.5	15.24±1.3	3.12±0.14	197.2±4.4
		99.45	98.23	99.04	98.96	98.11	96.19
				Fraction 2			
Certified Values	Modified BCR	3.77±0.28	45.70±2.0	124.0±3	26.60±1.3	126.0±3.0	114.0±5.0
This work	SEM	3.75±0.21	45.23±1.3	123.7±1.3	26.48±1.7	124.8±2.6	111.4±8.3
		99.47	98.97	99.75	99.54	99.04	97.72
	USE	3.59±0.33	43.66±2.1	122.6±2.6	25.80±1.4	124.4±1.3	111.1±9.2
		95.22	95.53	98.87	96.99	98.73	97.45
	MSE	3.72±0.14	44.37±1.9	121.3±2.6	26.32±2.2	119.2±3.1	108.8±4.7
		98.67	97.08	97.82	98.94	94.60	95.43
				Fraction 3			
Certified Values	Modified BCR	0.27±0.06	143.0±7.0	55.20±4.0	15.30±0.9	9.30±2.0	45.70±3.4
This work	SEM	0.26±0.02	141.1±9.2	54.16±5.2	15.06±1.3	9.11±1.8	44.26±2.2
		96.83	98.67	98.11	98.43	97.95	96.84
	USE	0.22±0.03	138.1±6.9	51.88±3.7	14.91±1.1	8.72±2.5	42.81±5.0
		81.48	96.57	93.98	97.45	93.76	93.67
	MSE	0.26±0.02	140.2±6.6	53.88±4.0	15.00±1.7	8.92±1.4	42.92±3.1
		96.36	98.04	97.60	98.04	95.91	93.91

***Mean±SD**

4.3.1- Ultrasonic bath assisted extraction in CRM 701

To compare the Ultrasound method with the conventional shaking procedures, the six replicate of BCR 701 were extracted according to conventional and ultrasound assisted procedures. The results are shown in **Figure 4.18**.



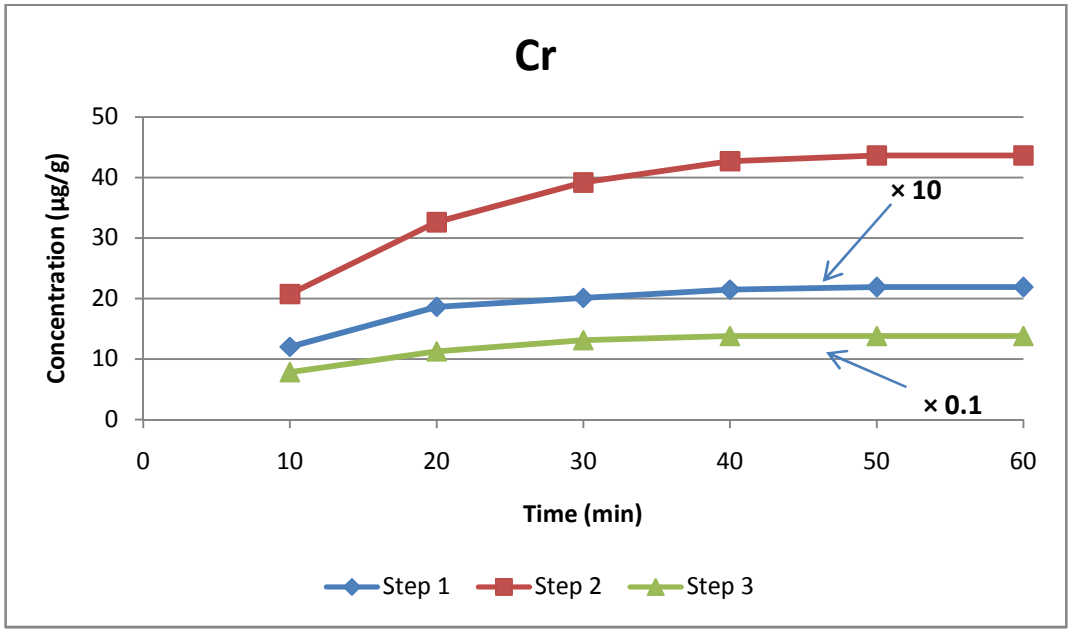
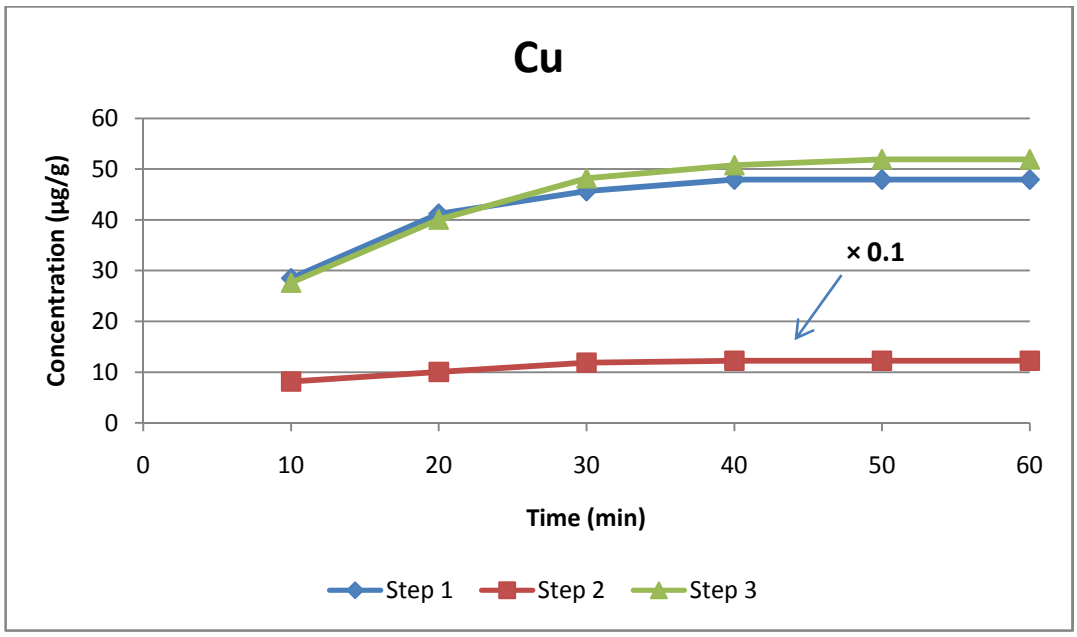
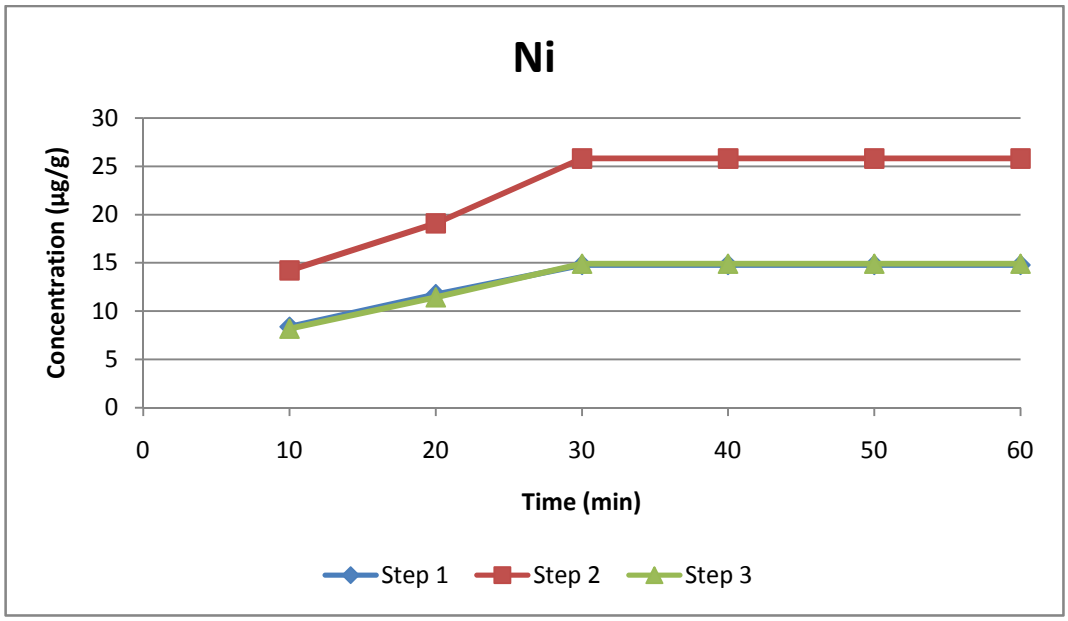
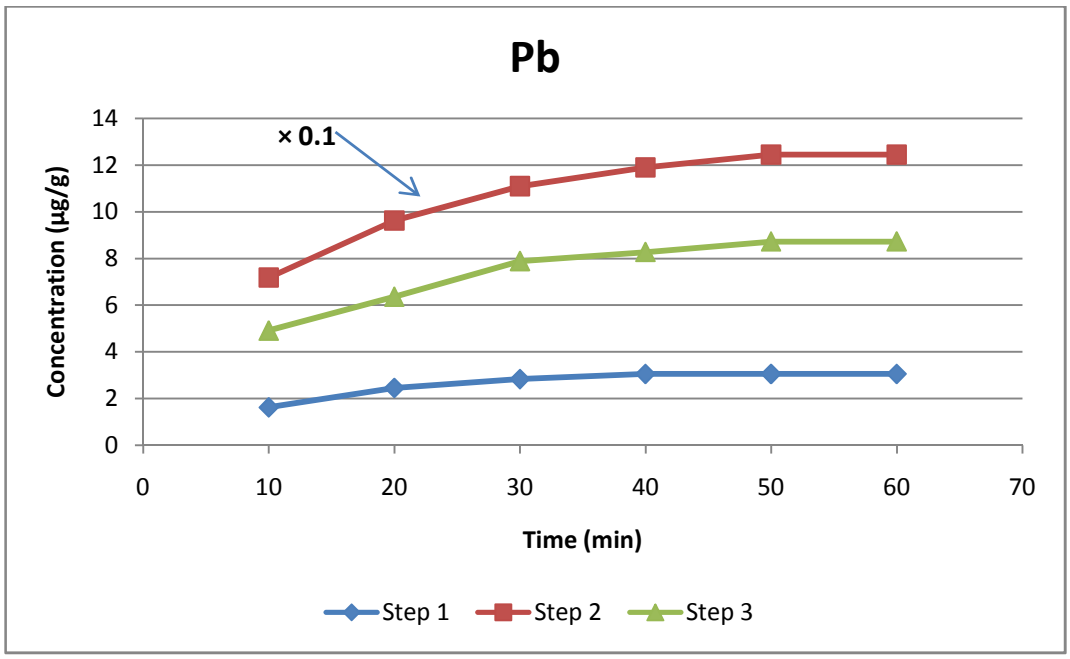


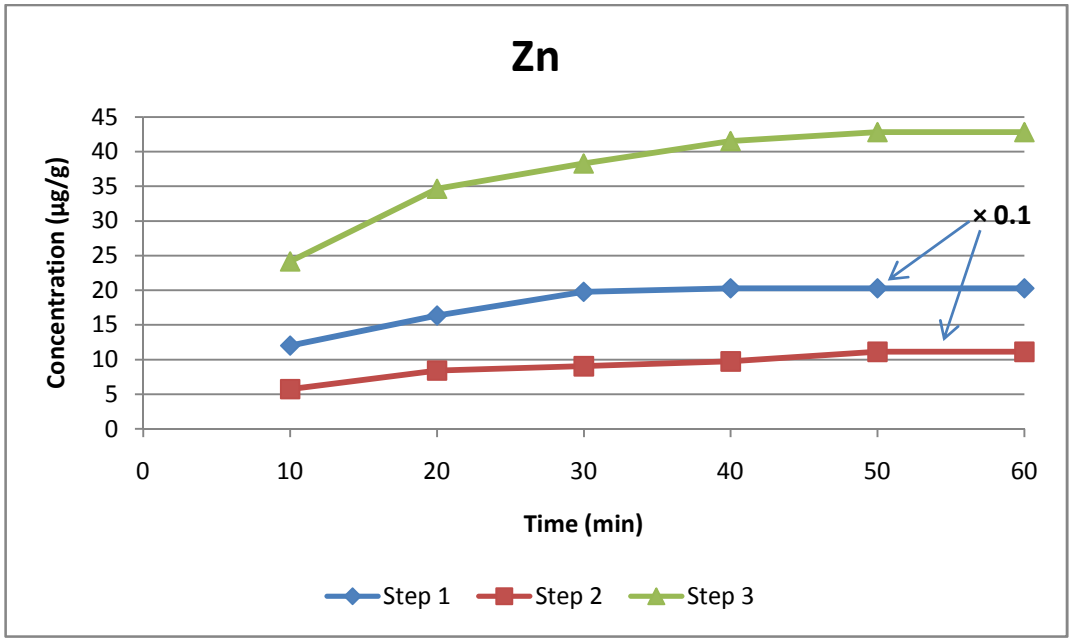
Figure 4.18: Concentration of elements by ultrasound extraction in CRM 701


Cu
Concentration (μg/g)
60
50
40
30
20
10
0
× 0.1
0
10
20
30
40
50
60
Time (min)
Step 1
Step 2
Step 3


Ni
Concentration (μg/g)
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Time (min)
Step 1
Step 2
Step 3

(Continued)


Pb
× 0.1
Concentration (µg/g)
Time (min)
Step 1
Step 2
Step 3


Zn
× 0.1
Concentration (µg/g)
Time (min)
Step 1
Step 2
Step 3

(Continued)

4.3.1.1- Comparison of the ultrasound and conventional extraction result on CRM 701

In ultrasonic bath of capacity 4 L, several samples can be proceeding at once. In our experiment, the temperature of ultrasonic bath was fixed at 30 ± 2° C by cooling the water of bath. After optimizing the parameters of the sonicator, the sequential extraction experiment was carried out on CRM 701. Each stage of ultrasonic sequential extraction procedure was characterized individually. The results found from each step of the extraction with the use of sonication at different time intervals, up to 60 min are shown in **Figure 4.18**.

Table 4.17 summarize the data for six heavy metals in CRM 701 by conventional, ultrasonic and microwave sequential extraction schemes. As you can see here the results of ultrasonic method were comparable with those obtained by conventional method. **Table 4.17** shows the recoveries for all elements in fractions 1 and 2 are more than 95 % and if compare with conventional method it will be acceptable. Cr and Ni have a noticeable recovery in Fraction 3. The lowest recovery was obtained for Cd in fraction 3. Other elements have the recovery values more than 93% too.

In fraction 1 Cu, Pb and Zn showed the highest recoveries in 40 min, while, for Cd , Cr and Ni it was obtained in 50, 50 and 30 min respectively.

Fraction 2 showed the highest recoveries for Cd, Cr, Pb and Zn in 50 min but Cu was found in 40 min and Ni in 30 min.

In fraction 3, the highest recoveries were found for Cu, Pb and Zn in 50 min while, for Cd and Cr found in 40 min and Ni in 30 min.

4.3.2- Microwave assisted extraction in CRM 701

The results obtained from heating the samples using a microwave oven (1000 W), at different heating time intervals (20-120 s) were compared with those values obtained by conventional modified sequential extraction method. It was observed that in fraction 1 the highest recovery were found for Cr, Ni and Zn at 70 s while the highest recovery obtained for Cd, Ni and Pb at 100 s. (**Figure 4.19**)

All recoveries have been obtained more than 98% except for Zn that is about 96 %. All recoveries were found more than 98 % for modified BCR method.

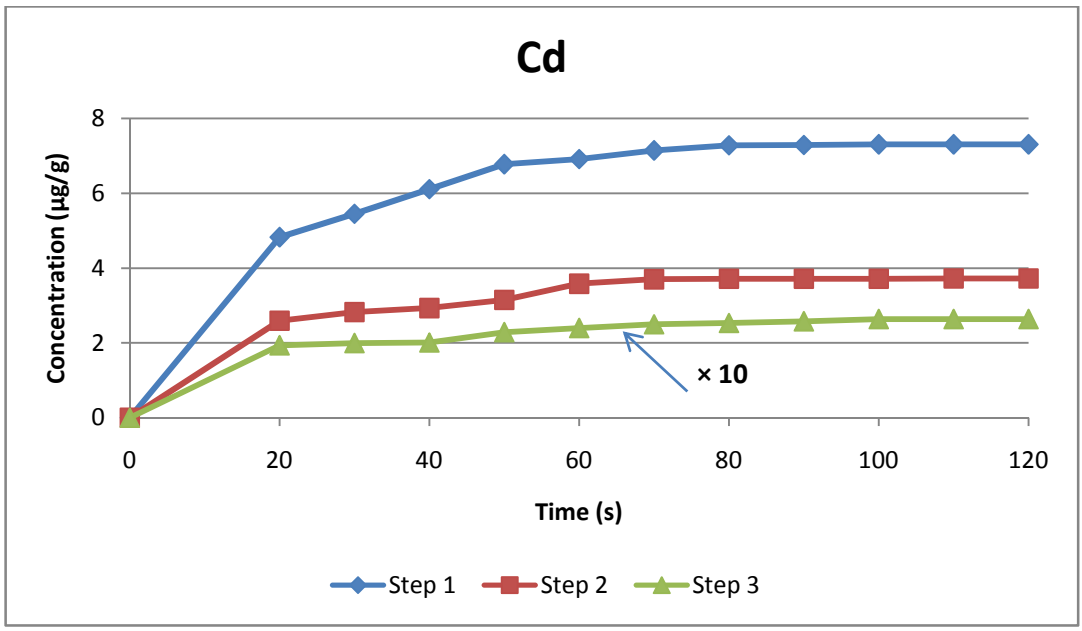
In fraction 2 of CRM 701 digested samples, the highest recovery have been achieved for Cr, Cu, Ni, Pb and Zn at 90 s but for Cd was found at 110 s.

The highest recoveries were obtained for all metals more than 95 % except for Pb which was about 94 %.

In this case, the highest recoveries were obtained for modified BCR more than 97 %.

In fraction 3, Cd, Cu and Pb showed the highest recoveries at 100 s, Zn and Ni at 60 s and Cr at 80 s. In this fraction the lowest recoveries were obtained for Pb and Zn similar to the Fraction 2.

The results are summarized in **Figure 4.19**.



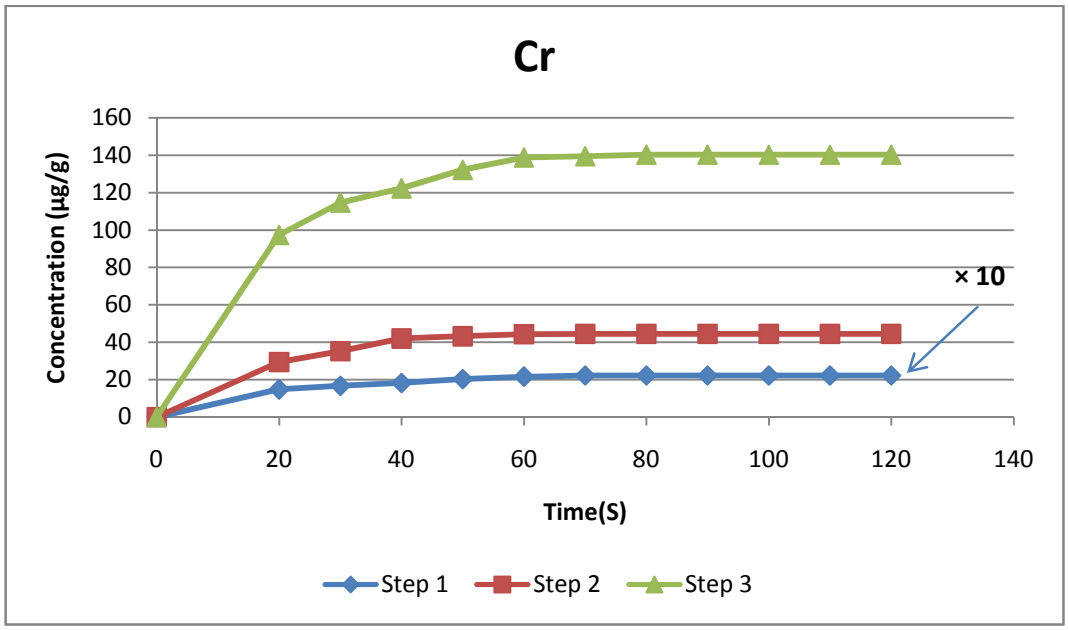
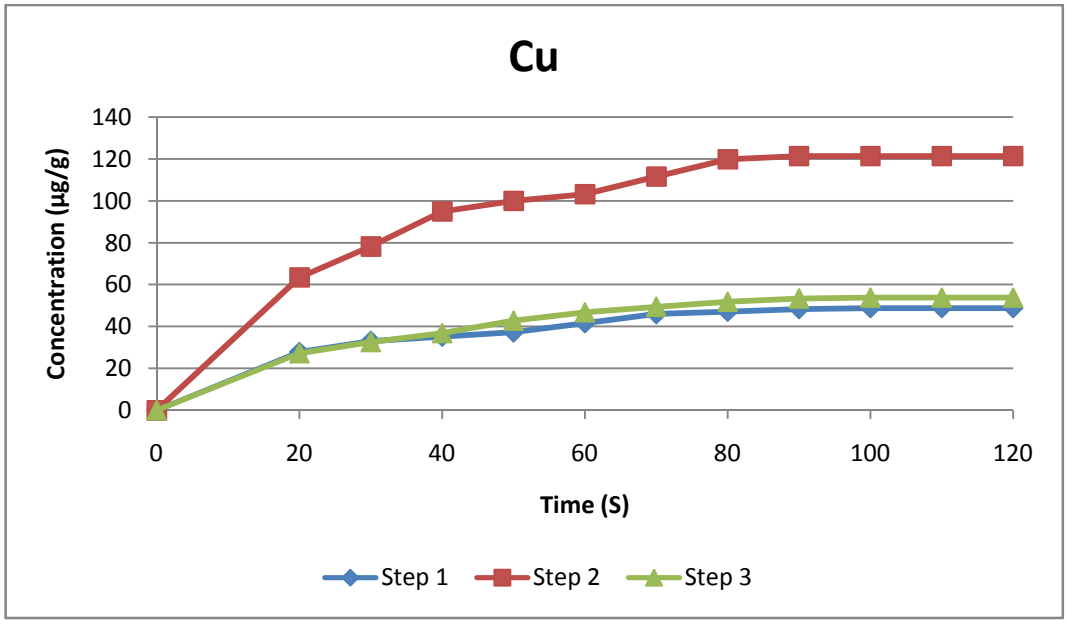
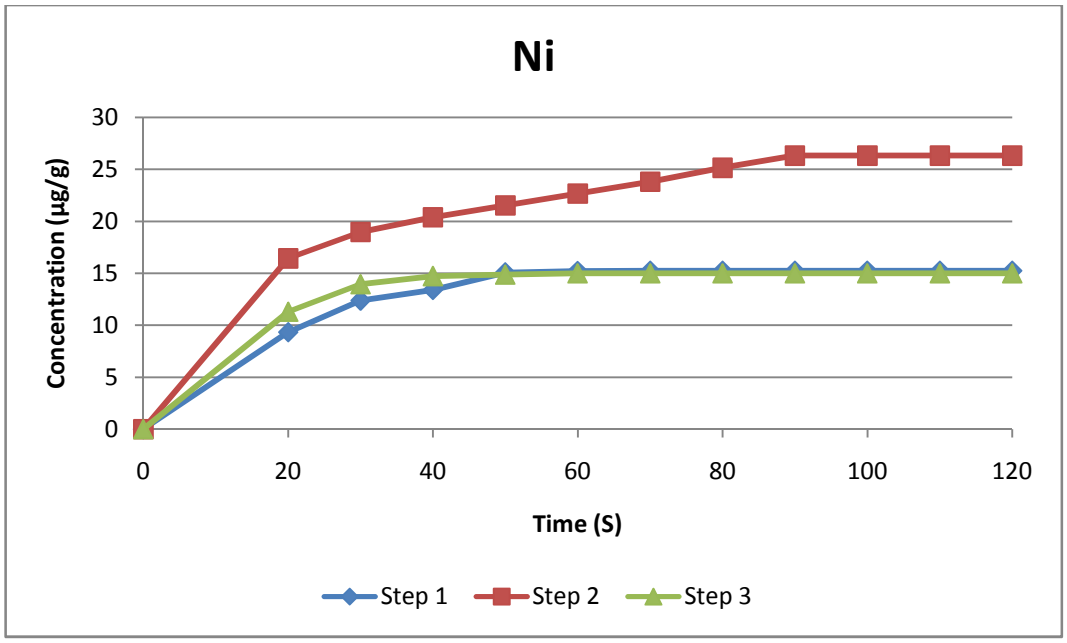
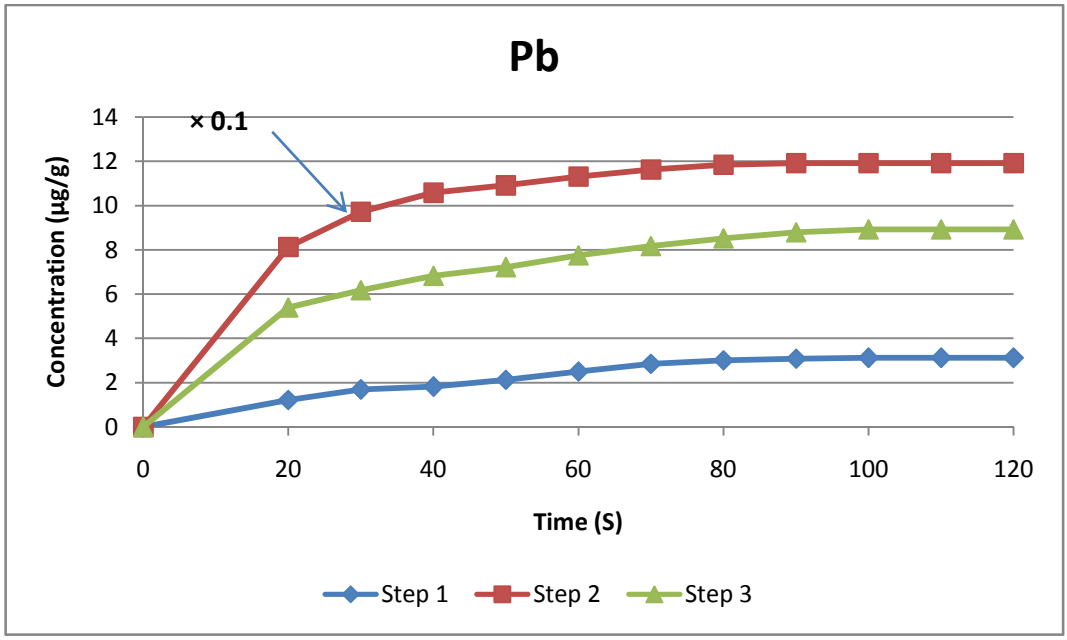


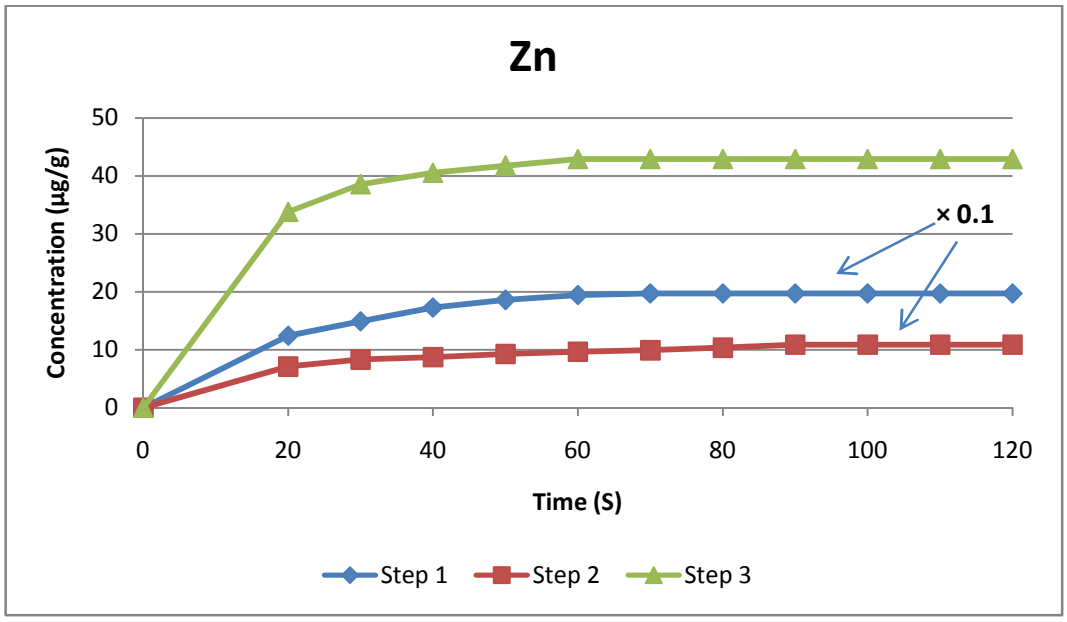
Figure 4.19: Concentration of elements by Microwave extraction in CRM 701


Cu
Concentration (µg/g)
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (S)
Step 1
Step 2
Step 3


Ni
Concentration (µg/g)
30
25
20
15
10
5
0
0
20
40
60
80
100
120
Time (S)
Step 1
Step 2
Step 3

(Continued)


Pb
× 0.1
Concentration (µg/g)
14
12
10
8
6
4
2
0
0
20
40
60
80
100
120
Time (S)
Step 1
Step 2
Step 3


Zn
Concentration (µg/g)
50
40
30
20
10
0
× 0.1
0
20
40
60
80
100
120
Time (S)
Step 1
Step 2
Step 3

(Continued)

Chapter 5:

Conclusion

5-Conclusion

The applications of sequential extraction procedures to environmental samples provide relevant information about possible toxicity when the sludge or sediments are discharged into the environment. The concentration of heavy metals in different samples such as sludge and sediments was discussed using different sequential extraction schemes:

In this study, the concentration of metals in the aquaculture shrimp sludge was in the following order, in this study: Ca>Fe>Mn>Zn>Pb>Cr>Cu>Cd.

In the case of mobility potential, Cd and Mn showed relatively high mobility potential compared to the other metals. The results of two extraction Tessier and BCR methods were quite similar, However the percent of recovery in BCR method was better than five steps Tessier method. The contamination factor of Cd in the aquaculture shrimp sludge was higher than other heavy metals. High amounts of Ca and Fe prove that this sludge is suitable as a fertilizer but if we are going to use it as a fertilizer it is better we use it for fruitless plants.

The studies of the effect of concentration and pH changes in step 2 BCR sequential extraction procedure showed that with an increase in the concentration of hydroxylammonium chloride to 0.5 mol L^{-1} at pH=1.5 concentration of zinc released was the highest. However, the best results were observed for Cu when the unmodified BCR sequential extraction scheme was used. According to the results obtained in this study, both Zn and Cu in the studied samples are not very mobile. In spite of that, the concentration of copper and zinc in these sludge samples were not higher as compared to typical soil ranges. Therefore, the use of this sludge as a soil improver or fertilizer is suitable.

Both unmodified and modified BCR sequential extraction methods were employed to evaluate the possible mobility and bioavailability of heavy metals present is shrimp aquaculture sludge. By increasing the concentration of hydroxylammonium chloride in step 2 from 0.1 to 0.5 mol L^{-1} and reducing the pH of the extractant to 1.5, the amount of most heavy metals released is significantly increased (except Cu).

Recoveries for all heavy metals are acceptable in the range 96–105%. The highest pseudo total concentrations found in the sludge were Fe, Mn, and Zn. The greatest release from exchangeable/ acid extractable fraction in the aquaculture sludge was found to be Cd (47%) followed by Mn (44%). Therefore, the potential mobility of these elements is higher as compared to other elements. Furthermore, other elements (Zn, Fe, Cu, Pb, and Cr) are mainly associated to organic matter/sulphide and in iron–manganese oxide or residual fraction. So the mobility of these metals from shrimp aquaculture sludge is quite low.

Total trace (Zn, Cu, Cr, Ni, Pb and Cd) element concentrations in two different composts containing shrimp aquaculture were determined using microwave assisted digestion and open system digestion procedures with AAS analysis. Four different digestion procedures using various HCl, HNO_3 and HF combinations and volumes were tested for both samples.

The results obtained for trace elements recoveries (Zn, Cu, Cr, Ni, Pb and Cd) of the certified material (BCR 146-R) using microwave acid digestion yield a recovery of 88.7–95.5%, 95.7–98.8%, 82.1–95.65% and 87.7–97.2% for procedures A–D, respectively. Good results were observed in both systems digestion. Nevertheless, the recoveries obtained for all elements in microwave assisted digestion were better compared to open system digestion with HNO_3–HCl–HF at ratios of 6:2:2 for both systems digestions. This was further confirmed by derived from shrimp sludge compost samples.

In the Sungai Buloh and Selat Melaka samples for investigation of distribution trace elements in different depth, 7 samples were collected from seven stations along the river and estuary and analyzed by modified BCR method.

In fraction 1, the concentration of metals in the top sediments were the highest compared to other depth subsamples for most of the elements at the seven sampling sites except for Cr at S7 in 15 cm depth and Ni S3 which has a dominant increasing from top to down. Variation trends of concentration for each element in the seven sample cores were approximately similar. We can see a normal decreasing from top to down. The surface enrichment may be due to contamination deposited from the surface waters, which also indicates that the pollutions are posed in the recent years. This is because the pollution is always absorbed into top sediment at first, and then sinks into more deep positions by chemical exchange.

In fraction 2, higher concentrations were found in the top sediments for most of elements except for V at S1 and S4 and also As at S1, S3, S4, S5 and S7. It should be highlighted that much higher concentration of Pb, Cd, Cr, Ni and Zn have been seen at S2 and S3 in the top sediments in compare with other stations. Also Pb, Cd and Cu in Station 1 were investigable. We can see a exception at 15 cm depth for As at some stations and also Cr in Station 7 that it can be interpreted as a special contamination for As and Cr in a few years ago.

The variation of the concentrations of elements in fraction 3 was more complex than the two fractions. There was no evident rule that could be concluded from the results, but some useful information could be obtained. The concentration of all elements except V, As and Co in station 2 and 3 are higher than other stations. Also we can see these result in station 1 for Pb, Cd and Cu. Elements in the fraction 3 mainly bound to various forms of organic matter by complexation and of natural organic matter or bioaccumulation in

certain living organisms through different ways. So the variation of the elements in this fraction became more complex and irregular than that in the fractions 1 and 2.

The main limitations of sequential extraction procedures are that, they are extremely time-consuming, and are less used for routine analysis. This problem has also been noted and is replacing the conventional procedure by other alternatives, such as microwave (MW) heating and ultrasonic (US) shaking. So a comparative study has been done between Modified BCR method, Ultrasound method and Microwave assisted digestion on CRM BCR 701.

The highest recoveries have been obtained for Modified BCR method although the best results were found for Microwave assisted digestion too. Also the recoveries of ultrasound method were less than others. Nevertheless, this method also can be acceptable. The time consuming was decreased in this methods.

The mobility and immobility and thus toxicity of heavy metals in waste depend largely on their type of binding forms. Result compares the mobility potential of heavy metals in different forms. It was noticed that Cd has highest ability and susceptible to be released from the shrimp aquaculture sludge by the simple ion exchanged mechanism. It is seen that the chloride content in the leachate can bind with Cd and enhance its mobility in the solid phase. This result is agreement with other studies. (Tuzen et al. 2003).

In this time the concentration of heavy metals in both samples still are under toxic values but with increasing of the trace elements concentration from deeper layer to surface it showed in the early future some of this element will be in toxic range. The precision of the proposed BCR extraction methods (expressed as RSD%) was found in the range of (3.99–9.6%) for all metals.

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1. **K. Nemati**, N. K. Abu Bakar, M. R. Abas, (2009). Investigation of heavy metals mobility in shrimp aquaculture sludge-comparison of two sequential extraction procedures. **Microchemical Journal**, 91, 227-231.
2. **K. Nemati**, N. K. Abu Bakar, M. R. Abas, E. Sobhanzadeh (2009). A modification of the BCR sequential extraction procedure to investigate the potential mobility of copper and zinc in shrimp aquaculture sludge. **Microchemical Journal**, 92, 165-169.
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4. **K. Nemati**, N. K. Abu Bakar, M. R. Abas, E. Sobhanzadeh, K. H. Low, (2010). Comparative study on open system digestion and microwave assisted digestion methods for metal determination in shrimp sludge compost. **Journal of Hazardous Materials**, 182, 453-459.
5. **K. Nemati**, N. K. Abu Bakar, M. R. Abas, E. Sobhanzadeh, K. H. Low, (2011). Comparison of unmodified and modified BCR sequential extraction schemes for the fractionation of heavy metals in shrimp aquaculture sludge from Selangor Malaysia. **Environmental Monitoring and Assessment**, 176, 313-320.
6. **K. Nemati**, N. K. Abu Bakar, M. R. Abas, E. Sobhanzadeh, (2011). Speciation of Heavy Metals by Modified BCR Sequential Extraction Procedure in Different Depths of sediments from Sungai Buloh, Selangor, Malaysia. **Journal of Hazardous Materials**, 192, 402-410.

International Conferences Attended

1. **K. Nemati**, N. K. Abu Bakar, M. R. Abas, International Applied Science Conference, 3-4 June 2009, Johor Bahru, Malaysia.
2. **K. Nemati**, N. K. Abu Bakar, M. R. Abas, 10^{th} Asian Conference on Analytical science 2009 (ASIANLYSIS), 11-13 August 2009 Kuala Lumpur, Malaysia.
3. **K. Nemati**, N. K. Abu Bakar, M. R. Abas, 6^{th} Singapore International Chemical Conference (SICC -6) 15-18 December 2009, Singapore.